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Foreword

During the last two decades there has been a marked development of interest in the cancer problem as manifested by the number of workers attracted to the field and the increased support which has become available for the work. The resultant significantly larger volume of reports of fundamental investigations has emphasized the need of improved facilities for prompt publication. In response to this need three Foundations which are contributing largely to the support of cancer investigation have accepted the responsibility of launching this new journal in cooperation with the American Association for Cancer Research. The start of this venture is being financed principally by these three organizations. An Advisory Board has been organized consisting of the Chairman of the Editorial Committee of the journal and one representative each from The International Cancer Research Foundation, The Jane Coffin Childs Memorial Fund for Medical Research, The Anna Fuller Fund, and The American Association for Cancer Research. The International Cancer Research Foundation has agreed to accept title to the journal and to hold the copyright in its name.

The conduct of CANCER RESEARCH will be in the hands of an Editorial Committee made up of representatives of the several fields which are involved in cancer research. The present policy is to publish original reports of laboratory and clinical investigations, abstracts of papers on cancer research appearing in other Journals, and a bibliography with abstracts, of current clinical reports. The abstract section is a continuation of the valuable service which was rendered for a number of years by the American Journal of Cancer.

A number of articles which had been accepted for publication by the American Journal of Cancer have been transferred to CANCER RESEARCH with the consent of the authors except in a few instances where it has not been possible to establish contact with them under existing conditions. The date of this transfer is considered as the date of receipt by this journal.

It is hoped that this new publication will receive wide support from all of those interested in advancing knowledge of the cancer problem.

Experimental Liver Cancer in Rats and its Inhibition by Rice-Bran Extract, Yeast, and Yeast Extract*

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It is a well established fact that the incidence of primary cancer of the liver among Orientals is much higher than the incidence among Europeans and Americans.

Nagayo (9) made a statistical study of liver cancer among Japanese, Chinese, and Koreans. In Japan, primary liver cancer ranks third in frequency of occurrence in males (6.7 per cent), and sixth in females (4.6 per cent). In China, hepatic cancer ranks fifth in males (8.1 per cent), and eighth in females (0.56 per cent), while in Korea, it ranks third in males (9.8 per cent), and fourth in females (6.6 per cent).

De Leon's study on malignancy among the Filipinos (2) shows that tumor incidence among Filipinos is not very different from that of occidental countries except in the high percentage of primary carcinomas of the liver. Of the 1,502 cases of malignant tumors admitted to the Philippine General Hospital in 1910-14 and 1922-26, 95 or 6.32 per cent were primary liver cancer.

Pack and Le Fevre (16) analyzed 19,129 patients with neoplastic diseases admitted for diagnosis and treatment at the Memorial Hospital, New York City, during 1917-29. They found that primary hepatic cancers form 0.17 per cent of the admissions to this institution.

Not only the racial characteristics, but also the dietary habits of the East and West differ greatly. Many attempts have been made to prove that diet has some effect on the incidence of cancer, but only in recent years has scientific evidence succeeded in showing that the nature of the food consumed has some definite influence in carcinogenesis.

In 1935 Sasaki and Yoshida (17) experimentally produced liver cancer in rats by feeding o-amino-azotoluene, and in 1937 Kinosita (6) using dimethyl-amino-azobenzene, commercially known as butter-yellow,[1] produced liver cancer in a much shorter time. In both cases, rice was used as the basal food for the animals, to correspond exactly to the staple food of the rice-eating countries of the Orient. The use of rice in their experiment is very important because when wheat bread was given the production of liver cancer was markedly reduced (4, 14).

Several inhibitory substances were tested by Japanese workers in their research. Okada (13) in 1938 reported that daily ingestion of a small amount of rice-bran oil prevented the production of liver cancer in rats fed butter-yellow. In the same year, Kinosita (5) and Ando (1) found that the addition of yeast to the butter-yellow rice diet decreased the production of liver cancer. Nakahara, Mori, and Fujiwara (11) used dried beef liver as a supplementary food with butter-yellow rice and demonstrated inhibition of liver cancer in rats.

At present it is important not only to verify the findings of the Japanese workers, but also to isolate the substance present in rice-bran oil, yeast, and beef liver that inhibits the production of liver cancer.

In view of these facts it seems desirable to give an account of experiments in which primary tumors of the liver, hepatoma and cholangioma, were produced in a high percentage of rice-fed rats which received butter-yellow. The formation of these tumors was prevented by feeding rice-bran extract, yeast, or yeast extract.

Experimental

In the present series of experiments we used p-dimethyl-amino-azobenzene (No. 338, Eastman Kodak Co.), discovered by Kinosita to be highly active in producing liver cancer in rats. This substance was dissolved in olive oil in the proportion of 3 per cent.[2] Twenty cc. of this solution was evenly mixed with 1,000 gm. of coarsely ground, unpolished Texas rice (fresh crop); *i.e.*, 0.6 gm. butter-yellow per kg. The rats were allowed to take as much of the mixture as they desired. The basal diet was supplemented with a small slice, about 1 gm., of fresh carrot per rat daily. Unlimited water was allowed.

As a preliminary study, the susceptibility of 3 different strains of rats to the carcinogenic action of butter-yellow was determined. Previous work had not taken into consideration the possible variability of the experimental animals.

Kinosita and other Japanese workers used albino rats of a mixed stock. In the present study we used young adult male and female rats of 3 pure strains, Wistar, Sherman, and Evans stock. They were 2 to 4 months

* Read at the Thirty-third Annual Meeting, American Association for Cancer Research, Pittsburgh, Pa., March 19-20, 1940.

[1] Butter-yellow was synthesized by Griess in 1875. It has been used to color oils, oleomargarine, and other vegetable fat substitutes for butter. In recent years the use of it in foods has been prohibited. However, Yellow A. B. (1-phenylazo-2-naphthylamine), and Yellow O. B. (1-o-tolylazo-2-naphthylamine), oil-soluble coal tar dyes are permitted in this country. These substances are somewhat related in structure to butter-yellow and the possibility of their acting in an analogous manner to butter-yellow cannot be overlooked.

[2] The 3 per cent butter-yellow solution in olive oil is a saturated solution. The butter-yellow readily dissolves in olive oil in this per cent when warmed on a water bath.

old, weighing 120 to 190 gm. at the beginning of experiments.

The amount of food consumed by each rat was carefully noted in order to calculate the approximate amount of butter-yellow ingested. A record of each animal was made, including the general physical condition, its weight at frequent intervals, and the duration of life, until death or until the animal was sacrificed.

Animals were killed at various stages from the beginning of butter-yellow feeding up to the development of advanced hepatic cancer, and the livers of these animals were examined grossly and microscopically.

The results obtained from this study are summarized in Table I and presented in graphic form in Fig. 7. This study included 8 groups of experiments, involving a total of 144 rats of the Wistar stock, 146 rats of the Sherman stock, and 132 rats of the Evans stock.

At the end of 2 months of butter-yellow feeding, the livers had irregular surfaces with more or less well defined nodules (Fig. 1.B). Histologically there was extensive proliferation of the fibrous connective tissue with lymphocytic infiltration, forming islands of hyperplastic liver cells (annular cirrhosis) (Fig. 2.B). Some nodules at this stage showed adenomatous hyperplasia of bile ducts with beginning malignant transformation into a cholangioma (Fig. 3).

At the end of 3 months of butter-yellow feeding, the livers contained nodules reaching 8 mm. in diameter. Some of the nodules were strikingly grayish white or yellowish in color on the reddish brown background of the whole organ (Fig. 1.C). Microscopic sections of the liver showed proliferation of the liver cords into papillomatous projections in acinar spaces giving the appearance of bile ducts in some areas (cholangioma type) (Fig. 4.A,B). Other sections of the liver showed liver cords proliferating into trabeculae with irregular

TABLE I: COMPARISON OF LIVER CHANGES IN RATS OF VARIOUS STRAINS MAINTAINED ON BUTTER-YELLOW RICE DIET *

Strain	Days																	
	1-24		25-40		41-60		61-75		76-90		91-120		121-150		151-180		181-220	
	Liv. cancer	No liv. cancer	Liv. cancer	No liv. cancer	Liv. cancer	No liv. cancer	Liv. cancer	No liv. cancer	Liv. cancer	No liv. cancer	Liv. cancer	No liv. cancer	Liv. cancer	No liv. cancer	Liv. cancer	No liv. cancer	Liv. cancer	No liv. cancer
Sherman	0	8	0	12	3	13	16	14	25	4	24	0	22	0	5	0	..	..
Wistar	0	7	0	14	6	16	10	10	12	5	21	4	14	0	18	0	7	0
Evans	0	5	0	14	0	22	0	10	2	9	11	11	12	4	11	2	19	0

* The sum of the numbers in the two columns under each period of days is the total number of animals of each strain killed for examination in that period.

All diagnoses were confirmed by microscopic examination.

The present results confirm the findings of Kinosita in every respect. The liver cancers were found to be various histological types of hepatomas as follows: trabecular or solid type; glandular or cholangioma type; and mixed type. Cirrhosis with adenomatous hyperplasia of bile ducts preceded these malignant tumors. Since the histology of these experimental liver cancers has been well described by Kinosita (6) and later by Nakahara, Mori, and Fujiwara (12), it seems unnecessary to enter into details. However, in view of the interest which has developed recently as to the possibility of a dietary factor as a causation of certain types of cancer in man, we felt it would be of interest to summarize our findings.

During the first 30 days' feeding on butter-yellow rice diet, the livers of rats showed no great changes in size, color, or shape. (Fig. 1.A). However, microscopic sections showed swelling of cells, much narrowing of sinusoids, and hyperchromatic appearance of nuclei. The surface of the capsule was smooth (Fig. 2.A).

and hyperchromatic nuclei (hepatoma type) (Fig. 5. A,B).

At the end of 5 months of butter-yellow feeding, the livers showed large, isolated, massive nodules measuring about 3 cm. in diameter. There were usually 1 or 2 such nodules in each lobe. Some of the nodules were strikingly whitish in color and soft; others were hemorrhagic and cystic (Fig. 1.D). Microscopic sections showed solid hepatomas in which the liver cells were enlarged; mitotic figures were present; and giant hyperchromatic nuclei were frequent (Fig. 6).

As may be seen from the data in Table I and Figure 7, the susceptibility of rats of different strains to the carcinogenic action of butter-yellow is not the same. Among rats of the Evans strain, the first liver cancer was noted on the 81st day after the beginning of the experiment. In the case of the Wistar strain the first liver cancer occurred in a rat dying 52 days after the beginning of the experiment, and in the case of the Sherman strain, the first tumor appeared after 49 days.

In the case of the Evans strain 2 out of 13 rats, or 15 per cent, living between 151 to 180 days did not

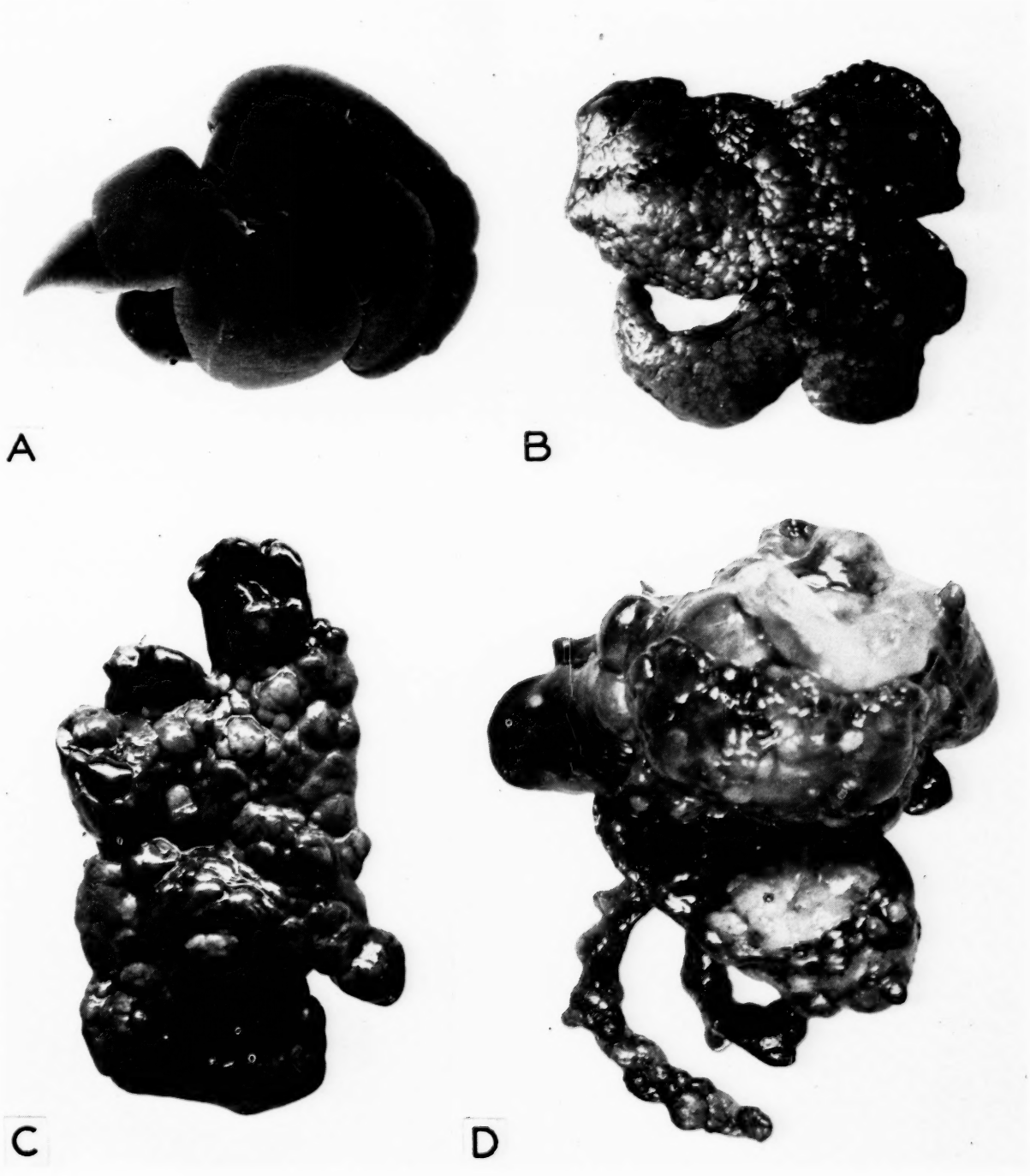

FIG. 1.

Gross appearance of livers of rats fed on butter-yellow rice diet for various lengths of time. Photographs of the livers were made at the same magnification, × 0.9.

A. Liver of a rat (30 days), showing practically normal gross appearance.

B. Liver of a rat (60 days), showing nodular cirrhosis.

C. Liver of a rat (90 days), showing hepatoma-nodules.

D. Extremely large liver of a rat (150 days), showing hepatoma-nodules and mesenteric metastasis.

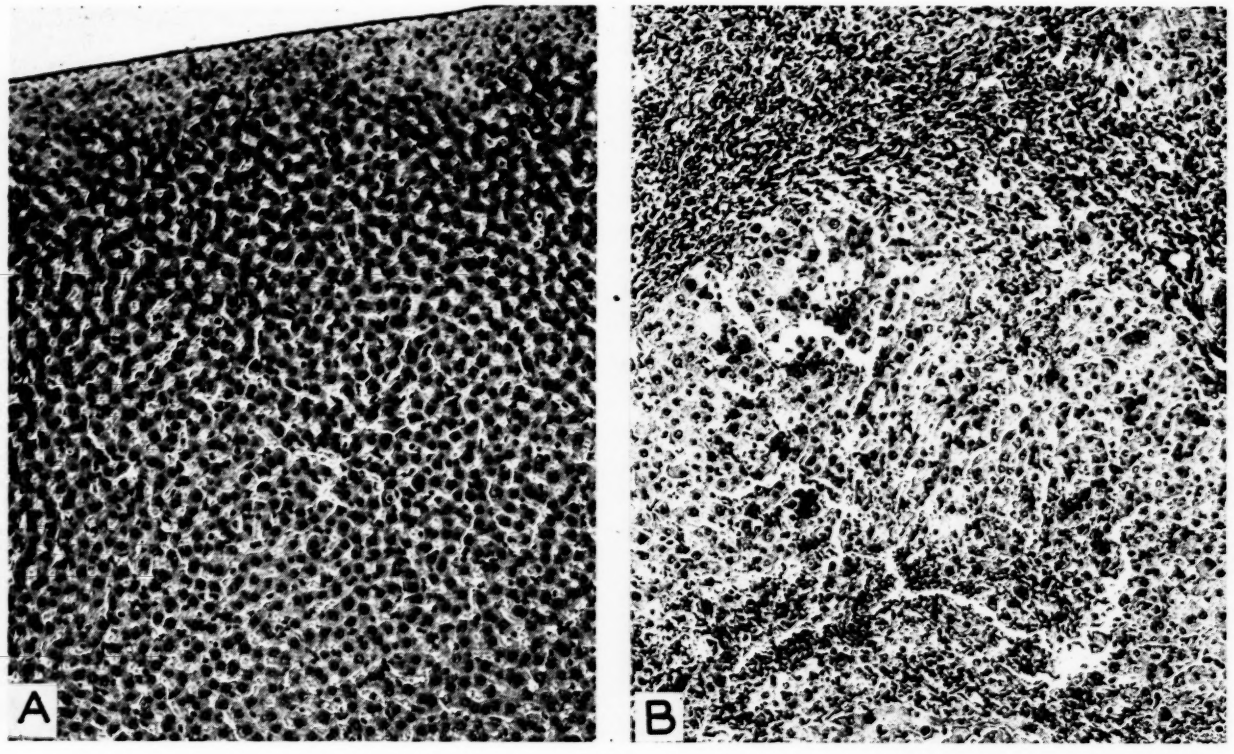

FIG. 2.

A. Section of a practically normal liver (30 days on butter-yellow rice diet), showing swelling of cells, much narrowing of sinusoids, and smooth surface of the capsule. (For gross liver see Fig. 1.A.)

B. Section of a cirrhotic liver (60 days), showing annular cirrhosis with marked lymphocytic infiltration. (For gross liver see Fig. 1.B.)

develop liver cancer, but showed liver cirrhosis. All of the 19 rats surviving 181 to 220 days of butter-yellow feeding showed liver cancer.

In the case of the Wistar and Sherman strains, all the rats that were maintained upon the butter-yellow rice diet for 124 days and 92 days, respectively, developed typical liver cancer.

Of the 185 rats from the 3 strains which died or were sacrificed between 91 and 220 days after the beginning of the experiment 164, or 89 per cent, had liver cancer and the other 21, or 11 per cent, showed liver cirrhosis with or without bile duct adenomatosis. Of the 101 rats of the Wistar and Sherman strains which died or were sacrificed between 100 and 200 days after the beginning of the experiment 98, or 97 per cent, showed liver cancer; and the other 2, or 3 per cent, showed liver cirrhosis with or without adenomatous hyperplasia.

Under the conditions of our experiments only about 3 per cent of the rats died during the first month without noteworthy liver change, and about 15 per cent died during the first 60 days of feeding. The low mortality rate in our rats is surprising because Harada, Mizuta, and Maruya (3) at Kinosita's laboratory found 45 per cent mortality in their rats during the first month. Similarly, Nakahara and co-workers (12) reported that many of their butter-yellow fed rats died

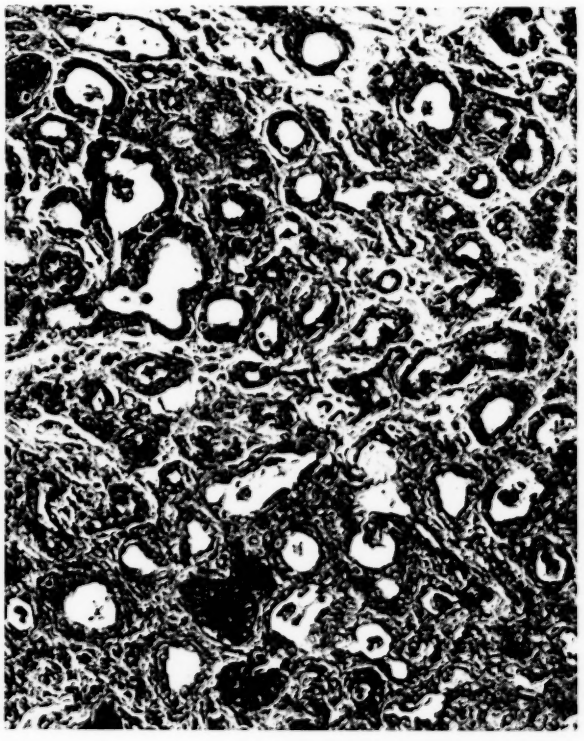

FIG. 3.

Section of a liver (60 days), showing bile duct adenomatosis with beginning malignant transformation into a cholangioma.

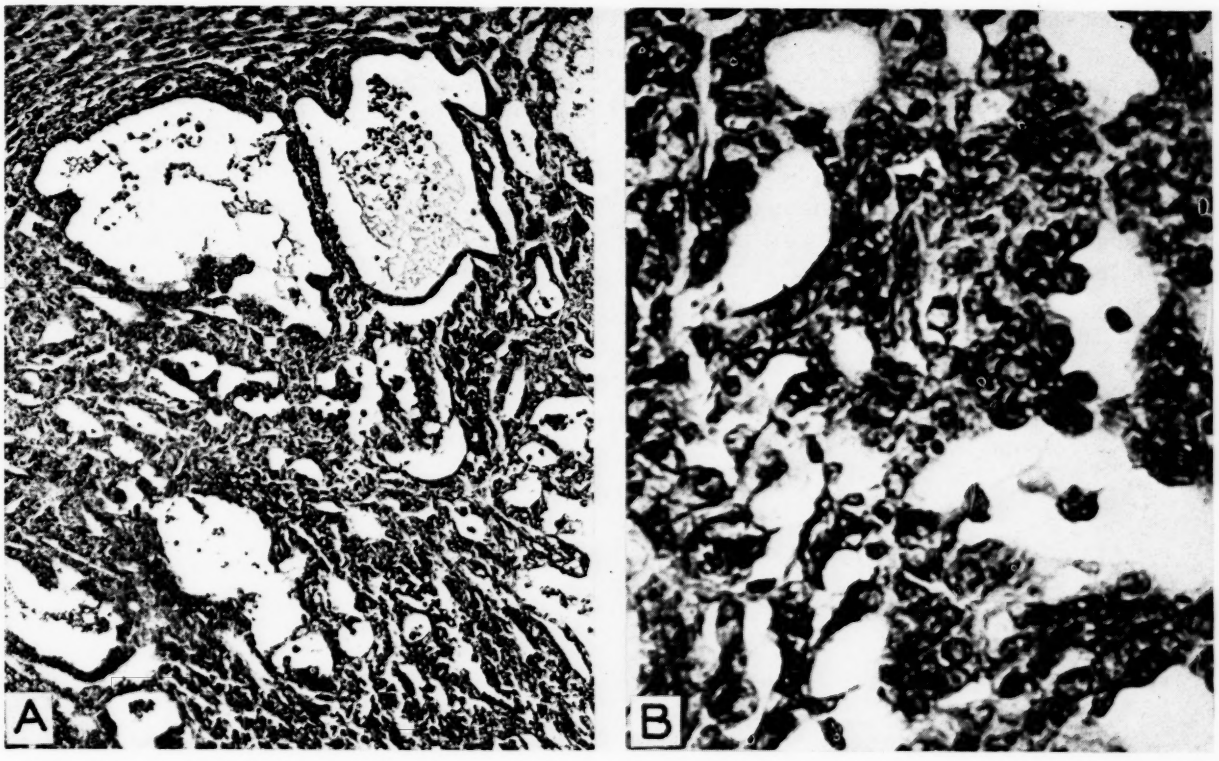

FIG. 4.

A. Section of a liver cancer (90 days), showing cholangiomatous type of tumor. (For gross liver see Fig. 1.C.)
B. Same section at higher magnification.

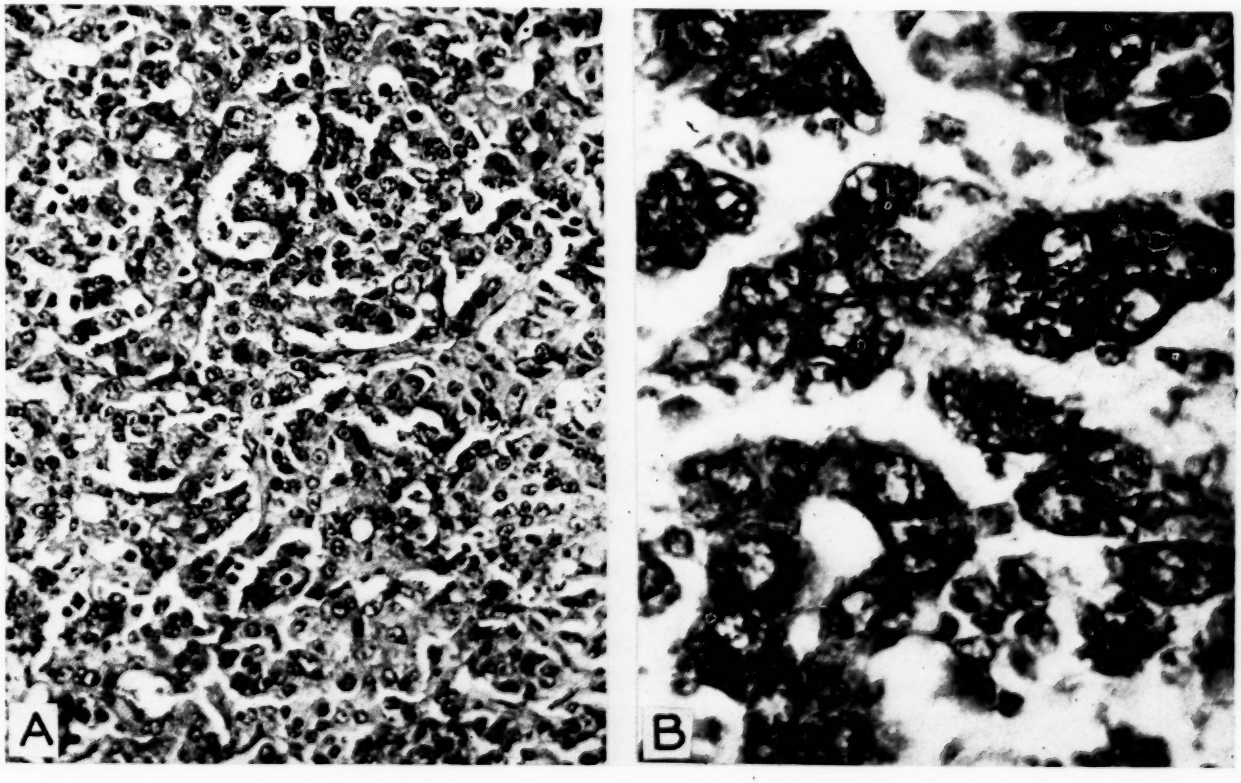

FIG. 5.

A. Section of a liver cancer (hepatoma) (90 days), showing trabecular type. (For gross liver see Fig. 1.C.)
B. Same section at higher magnification.

early without showing any significant change in the liver.

Although the number of days of feeding was the same, individual rats consumed different amounts of butter-yellow. Therefore, we calculated approximate amounts of butter-yellow consumed by each animal from a large number of rats and found about 162 mgm. of butter-yellow for the first 50 days and 120 mgm. for the second 50 days, or a total of 282 mgm. for 100 days.

Nutrition of young adult rats, of the Wistar and Sherman strains, during ingestion of the butter-yellow rice diet with supplement of fresh carrots, may be briefly summarized as follows: During the first 10 days individual animals consumed about 8 gm. of the basal diet with butter-yellow daily, during the next 20 days about 5 to 6 gm., and during the second and third months about 4 gm. The majority lost body weight from the beginning of the experiment, at an average of about 0.5 gm. daily. Some gained weight slightly during the first 2 weeks, and maintained their weight until the onset of liver cancer. At the end of 2 months, when the livers of most of the rats were cirrhotic, the general appearance of the animals was good, but thereafter, when malignant changes began to appear in the liver, the condition of the animals was poor.

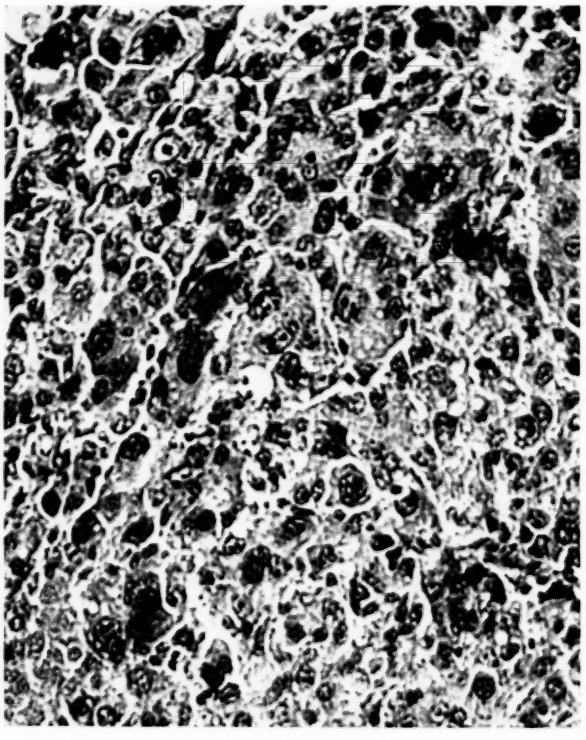

FIG. 6.

Section of a liver cancer (150 days), showing solid type of hepatoma. (For gross liver see Fig. 1.D.)

After the rats had been fed butter-yellow for 90 days, 2 types of liver with cancer were observed. One was very small, weighing about 2.1 gm., and the other of normal size, weighing about 8.9 gm. Livers of normal rats weighing about 150 gm. have an average weight of 8 gm. In some of the well-nourished rats living 150 days or more, the liver reached a huge size, weighing from 24 to 75 gm. The cancers occupied about 90 per cent of the organ (Fig. 1.D). Small livers showed more fibrosis or cirrhotic changes with comparatively few cancer nodules, while the normal or large livers contained more numerous multicentric patches of carcinoma and much less fibrosis or cirrhotic change. From these observations we are inclined to believe that the state of nutrition of the animal has much to do with the type of tumor which develops. In undernourished rats the livers were abnormally small and more cirrhotic, while in the well-nourished rats the livers were normal or very large with more cancerous nodules.

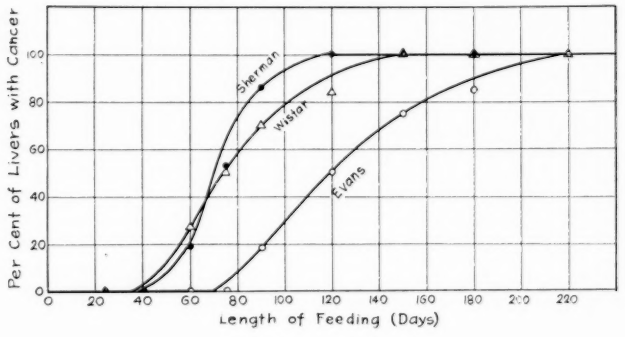

FIG. 7.

Curves showing percentage of liver cancer in rats of the Sherman, Wistar, and Evans strains maintained on a butter-yellow rice diet.

Among rats of the Sherman, Wistar, and Evans strains examined between 150 and 220 days of butter-yellow feeding, 6 out of 12 livers of Sherman rats, 16 out of 35 livers of Wistar rats, and 6 out of 37 livers of Evans rats were very large. Only among rats surviving for a longer period and exhibiting large livers were metastases found. Sixteen out of 84 rats had widespread metastases in the mesentery and omentum, and 4 of them also had lung metastases. Most of the metastases were of cholangioma type.

Ascites was found only among rats surviving 150 days or longer and exhibiting large livers (Fig. 1.D). Among 84 such rats 11 had ascites.

The spleens of rats fed butter-yellow rice diet for about 65 days were greatly enlarged in size. This splenomegaly coincided with the development of liver cirrhosis. Many of the spleens weighed 2 to 3 times the normal weight. However, with the development

of the liver cancers the enlarged spleens gradually returned to normal size.

None of the 135 young adult female rats kept with male rats became pregnant while on the diet. On the other hand, 10 mated female rats maintained upon a ration of unpolished rice and carrots produced 1 to 3 litters of young during an experimental period of 200 days. This is in accord with the observation of Kusumoto (8) who noted ovarian atrophy in these animals.

Effect of Feeding Carrot

In the preceding experiments we supplemented the basal diet of butter-yellow and rice with a small slice of fresh carrot per rat every day in order to maintain the normal metabolism and maintain the normal physical condition of the animal. The carrot is rich in vitamin A.

Table II: Effect of Absence of Carrots on the Production of Liver Cancer by Butter-Yellow

Rat no.	No. of days survived	Liver findings
1	12	Normal
2	60 *	Cirrhosis
3	61	Cirrhosis
4	65	Cirrhosis
5	69	Cholangioma
6	75	Cholangioma
7	77	Cholangioma
8	77	Cholangioma
9	83	Cholangioma
10	85	Cholangioma
11	86	Cholangioma
12	90 *	Cholangioma
13	90	Hepatoma
14	97	Hepatoma
15	107 *	Cholangioma
16	112	Cholangioma, Hepatoma

* Indicates the animal was sacrificed and examined.

The following experiment records the results obtained with 16 rats of the Sherman strain fed on the butter-yellow rice diet without the supplement of carrots.

The data in Table II show that young adult rats maintained upon the butter-yellow rice diet alone died definitely earlier than those maintained upon a similar diet with the supplement of carrots (Table I). The animals without carrots continued to lose weight until death.

In the livers of these animals there were numerous small nodules with a few large ones. Histological examination revealed that about 75 per cent of these liver cancers were cholangiomatous in type. When the basal diet was supplemented with carrots the animals lived longer. The livers grew larger and showed many large cancer nodules, mostly of the hepatoma type. It is clear from this finding that daily ingestion of a small slice of fresh carrot has no noticeable effect on the production of liver cancer by butter-yellow, but it influences the survival of the animal and consequently the type of liver cancer.

Effect of Feeding Rice-Bran Extract

Before the Third International Cancer Congress at Atlantic City, September 1939, Kinosita (7) stated that the production of liver cancer by butter-yellow is stimulated by feeding with polished rice instead of unpolished rice, but is slightly inhibited by feeding with bread. When 1 cc. of rice-bran oil (ether soluble substance) is given daily to the rat the production of butter-yellow cancer is also definitely hindered.

Kinosita obtained rice-bran oil by pressing the rice-bran from fresh crop rice (Fall harvest rice) at a temperature of 60 to 70° C. In our study the same kind of rice-bran oil was prepared. Rice-bran from a fresh crop of Texas rice was continuously extracted for 24 to 48 hours with ether free from H_2O_2. From 1,800 gm. of rice-bran, about 400 cc. of a greenish brown oily emulsion was obtained. The extract is supposed to contain vitamins A, B_1, and E. Vitamin B_2 was present in a very small amount.

The vitamin A content of the rice-bran extract was tested by feeding 10 young albino rats upon a synthetic diet (19) consisting of casein, 18 per cent; cane sugar, 10 per cent; starch, 36 per cent; salt mixture, 3 per cent; yeast, 3 per cent; lard, 20 per cent; and rice-bran extract, 10 per cent. After 3 months, xerophthalmia failed to appear in these animals and their physical appearance was normal.

Tests for vitamin B_1 were made on 4 pigeons. When these animals developed symptoms of polyneuritis upon feeding with polished rice, the administration of 1 to 2 gm. of the rice-bran extract to each bird caused a disappearance of the beriberi symptoms within 10 hours. The pigeons remained normal for several days upon continued feeding with polished rice alone.

Vitamin E content was assayed by feeding young albino rats, 4 males and 6 females, upon a synthetic diet consisting of casein, 18 per cent; cane sugar, 10 per cent; starch, 26 per cent; salt mixture, 3 per cent; yeast, 3 per cent; butter fat, 30 per cent; and rice-bran extract, 10 per cent. Three of the 6 female rats gave birth to 1 to 2 litters of young during the experimental period of 100 days. This shows the presence of vitamin E.

The content of riboflavin, vitamin B_2, in the rice-bran extract was estimated by the fluorometric method. Spectroscopic analysis showed the presence of unsaturated hydrocarbons related to ergosterol, pro-vitamin D. The rice-bran extracts were kept in a refrigerator until used.

In this experiment we used 90 rats of the Sherman and Wistar strains. One group of 60 rats was maintained on the mixture composed of 96 per cent unpolished rice, and 4 per cent rice-bran extract, supplemented with a small slice of fresh carrot per rat daily. Thirty rats of the second group were kept on unpolished rice and fresh carrots, but without rice-bran extract.

TABLE III: EFFECT OF FEEDING RICE-BRAN EXTRACT AND UNPOLISHED RICE ON THE PRODUCTION OF LIVER CANCER BY BUTTER-YELLOW

Rat no.	Sex	No. of days fed	Body weight (gm.) Initial	Maximum	Final	Liver findings *
1	♂	100	134	157	157	—
2	♂	100	121	136	120	—
3	♀	100	125	145	142	—
4	♂	106	150	155	154	—
5	♂	110	147	177	170	—
6	♂	110	150	151	142	±
7	♂	112	115	122	122	±
8	♀	114	117	122	100	—
9	♂	116	119	136	133	—
10	♀	120	142	142	127	—
11	♀	120	132	132	118	—
12	♂	120	137	137	70	±
13	♀	125	124	151	149	—
14	♀	128	121	121	100	—
15	♂	129	136	155	155	—
16	♂	130	126	126	108	—
17	♀	130	129	139	144	±
18	♂	138	122	144	144	±
19	♂	139	120	151	151	—
20	♂	139	132	132	122	—
21	♂	139	116	127	120	±
22	♂	149	148	148	130	—
23	♀	150	127	160	147	—
24	♀	150	117	128	120	±
25	♂	150	122	122	117	±
26	♀	153	145	145	109	—
27	♀	161	115	133	100	—
28	♂	161	159	159	120	—
29	♂	161	150	150	124	—
30	♂	161	167	167	165	—
31	♀	161	135	138	117	±
32	♂	161	151	151	110	+
33	♂	161	179	179	168	+
34	♂	170	158	165	161	—
35	♀	172	113	118	118	—
36	♂	185	140	140	120	±
37	♀	200	121	135	130	±
38	♀	200	122	129	140	+
39	♂	218	174	160	160	—
40	♂	218	133	133	95	±
41	♂	218	162	173	144	±
42	♂	218	144	150	138	++
43	♂	228	108	128	112	+
44	♂	250	140	140	135	—
45	♂	250	148	148	123	±
46	♀	250	135	149	149	±
47	♂	250	135	135	111	+
48	♀	250	141	173	162	++

* — indicates smooth, practically normal liver; ± indicates nodular cirrhosis with adenomatous hyperplasia; + indicates distinct areas of cholangioma or hepatoma, or both; ++ indicates extensive liver cancer with or without metastasis. In all cases the diagnoses were confirmed by microscopic examination.

Twenty cc. of 3 per cent butter-yellow solution in olive oil were mixed well with 1,000 gm. of unpolished rice, with or without rice-bran extract. In order to prevent possible deterioration, the food mixtures were kept in a cool place.

The results of the above experiments are recorded in Tables III and IV. Since we observed that the susceptibility of rats of the Sherman and Wistar strains to the carcinogenic action of butter-yellow is practically the same, the findings in both groups were combined.

Rats that died or were sacrificed before the period of 100 to 250 days are not included in Table III, experimental group. None of them, however, showed any malignant change. Similarly, animals of the control group that died or were sacrificed before 100 days are not included in Table IV, although many of them showed liver cancer.

TABLE IV: EFFECT OF UNPOLISHED RICE ALONE ON THE PRODUCTION OF LIVER CANCER BY BUTTER-YELLOW

Rat no.	Sex	No. of days fed	Body weight (gm.) Initial	Maximum	Final	Liver findings *
1	♀	100	145	145	130	+
2	♀	100	136	136	83	+
3	♂	105	153	154	117	+
4	♂	128	135	136	130	—
5	♀	128	118	118	75	+
6	♀	130	134	145	124	+
7	♂	134	179	197	136	+
8	♀	136	119	119	74	+
9	♂	141	139	139	125	++
10	♂	150	148	156	146	+
11	♀	176	135	136	92	++
12	♂	182	136	140	119	+
13	♂	182	141	141	110	+
14	♂	182	111	125	122	+
15	♀	182	114	114	64	+
16	♂	182	121	130	130	++
17	♀	182	134	138	115	++
18	♂	184	120	120	80	++
19	♂	188	167	167	165	+
20	♂	200	160	160	105	++

* See Table III for explanation of symbols.

From the data in Tables III and IV, it will be seen that rice-bran extract exerted a definite inhibiting effect upon the production of liver cancer in rats by the oral administration of butter-yellow. This inhibiting action was already demonstrated at a period between 100 and 150 days after the beginning of the experiment when 25 of the 60 rats fed with rice-bran extract were killed for examination. The livers of 17 rats had the normal smooth surface, while in the remaining 8 the surface was slightly granular. Microscopic sections of the smooth livers revealed only beginning connective tissue increase with slight regeneration of the bile ducts

(Fig. 8.A). Histologic examination of the granular livers, observed in rats kept on this diet for 4-5 months, showed proliferation of fibrous connective tissue inclosing islands of liver cords, resembling the cirrhotic condition produced in the liver of rats fed with butter-yellow rice diet without supplementary rice-bran extract for 2 months (Fig. 8.B). There was no evidence of malignant change in any of these granular livers. On the other hand, 9 out of 10 rats on butter-yellow rice diet without the supplementary rice-bran extract rather than a preventing action because some of the rats finally developed liver cancer. Thus, among 23 rats in this group surviving a period of 153 to 250 days 7, or 30 per cent, were found with typical liver cancer, and 7 others showed liver cirrhosis with adenomatous hyperplasia, a forerunner of malignancy. All control rats had liver cancer. The present result is in accord with the findings of Okada (15), who observed that daily ingestion of rice-bran oil delayed the development of liver cancer in rats fed with butter-yellow bread diet.

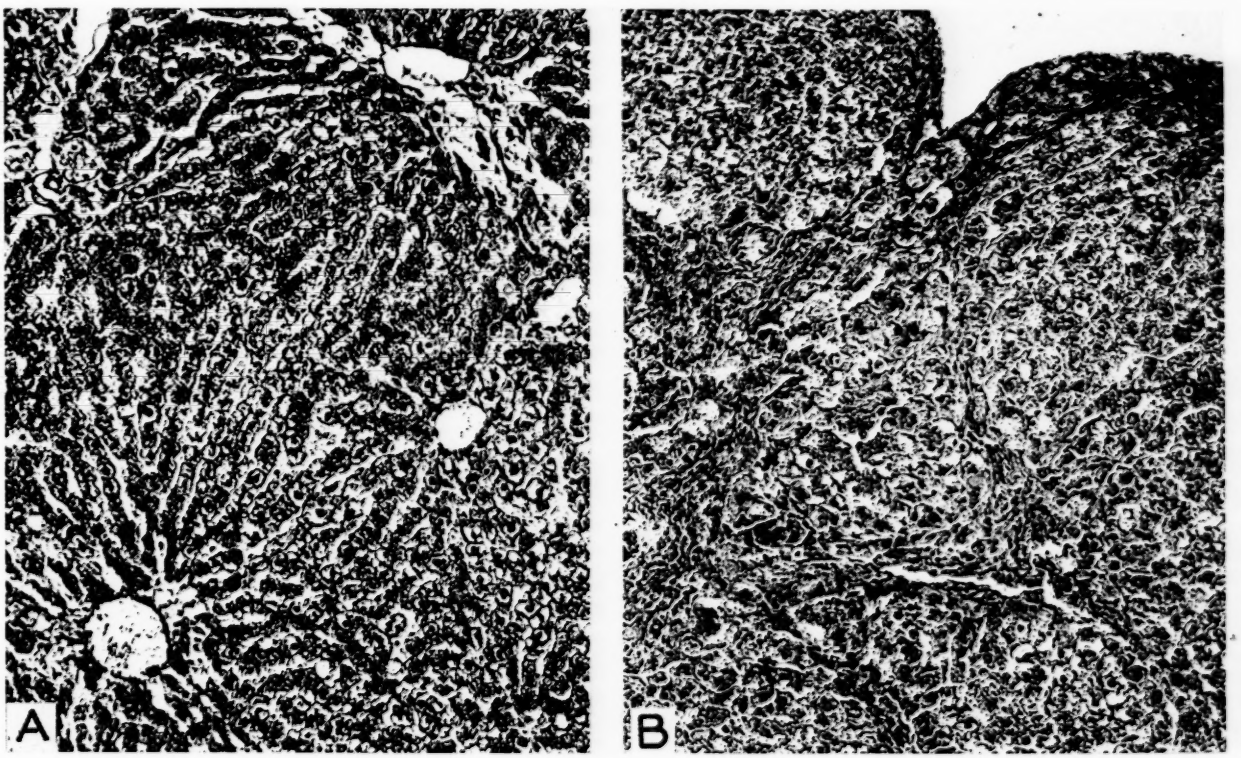

FIG. 8.

A. Section of the liver of a rat fed on butter-yellow rice diet supplemented with rice-bran extract for 150 days. The liver lobules are distinct with slight proliferation of connective tissue.

B. Section of the liver of a similarly fed rat (150 days), showing marked proliferation of fibrous connective tissue inclosing islands of liver cells (cirrhosis). No evidence of malignant change.

produced liver cancer in the same period of 100 to 150 days.

It was observed that rats fed on butter-yellow rice supplemented with rice-bran extract generally gained weight and presented a healthy appearance. Each rat consumed approximately 6 to 8 gm. of the food per day, an amount greater than that consumed by rats fed on butter-yellow rice alone. This observation is important because we are sure that the amount of butter-yellow ingested by each experimental rat during the first 100 days was much greater than 282 mgm., which is the average amount for a rat of the cancer control group.

The inhibiting effect of the rice-bran extract upon experimental liver cancer appears to be a delaying

Effect of Feeding Yeast Extract

It was shown in the above experiments that the ether extract of rice-bran contains a substance which is inhibitory to the development of liver cancer by butter-yellow feeding. The question then arose: Is the cancer-inhibiting substance a fat soluble substance or a water soluble substance, or both?

In an attempt to answer this question, the effect of an ether extract of yeast upon butter-yellow liver cancer was investigated. Recent studies of Kinosita (5), Ando (1), and Nakahara, Fujiwara, and Mori (10) showed that yeast feeding tends to inhibit the experimental production of liver cancer.

Following the same technic as that of rice-bran ex-

traction, dried brewer's yeast (No. 2040, Fleischmann Laboratories) was continuously extracted with ether for 24 to 48 hours. From 10,000 gm. of yeast 165 gm. of a thick brown-colored extract were obtained. It contained 14.5 γ of vitamin B_2, riboflavin, per gram of the material. It also contained ergosterol.

The experimental group of 30 rats was kept on the mixture containing 99.75 per cent of butter-yellow rice and 0.25 per cent of yeast extract, supplemented with a small slice of fresh carrot per rat daily.

TABLE V: COMPARATIVE EFFECTS OF FEEDING YEAST EXTRACT AND OF OMISSION OF YEAST EXTRACT ON THE PRODUCTION OF LIVER CANCER BY BUTTER-YELLOW

Group fed yeast extract			Group without yeast extract		
Rat no.	No. of days fed	Liver findings *	Rat no.	No. of days fed	Liver findings *
1	100	—	1	100	+
2	100	—	2	100	+
3	100	—	3	100	+
4	100	±	4	105	+
5	104	±	5	110	+
6	106	—	6	127	+
7	106	—	7	134	+
8	130	—	8	141	+
9	130	+	9	151	++
10	145	++	10	164	+
11	149	—	11	176	++
12	178	++	12	179	++
13	183	+	13	184	+
14	188	+	14	188	++
15	189	—	15	197	++
16	193	++			
17	200	—			
18	203	++			
19	208	—			
20	208	±			
21	208	±			
22	208	+			
23	208	+			
24	208	+			
25	208	+			
26	208	++			
27	219	+			
28	223	++			

* See Table III for explanation of symbols.

The control group of 20 rats was maintained on butter-yellow rice without yeast extract, but with fresh carrot.

The results are presented in Table V. Rats used in this study were of the Wistar and Sherman strains.

Table V shows clearly that the development of liver cancer in rats following oral administration of butter-yellow is inhibited by daily ingestion of a small amount, 18 mgm., of yeast extract. Among 28 rats of the experimental group examined after 100 to 223 days, 10 rats, or 36 per cent, showed a smooth normal liver, 4 rats, or 14 per cent, developed liver cirrhosis with adenomatous hyperplasia, and 14 rats, or 50 per cent, had cholangioma or hepatoma, or both. On the other hand, all of the 15 rats, or 100 per cent, in the control group examined between 100 and 197 days showed typical liver cancer.

The yeast extract protected 33 per cent of the animals surviving more than 200 days from the carcinogenic action of butter-yellow.

Animals fed on the butter-yellow rice diet with the supplement of yeast extract presented a much healthier appearance and lived much longer than the control animals. In the experimental group, rats Nos. 4, 8, 13, 14, 16, 18, 27, and 28 became weak and emaciated. Some of these animals died and others were sacrificed when it became apparent that they would not survive. The remaining 20 rats were sacrificed for examination. In the control group, 12 rats died or were killed because of their terminal condition. Rats Nos. 6, 13, and 15 were sacrificed for examination.

EFFECT OF FEEDING 15 PER CENT WHOLE YEAST

Recently Nakahara, Fujiwara, and Mori (10) reported that the addition of yeast to a rice diet decreased the rate of liver cancer production. The experiment on which this conclusion was based follows:

> One group of 57 albino rats was maintained on unpolished rice with the occasional addition of fresh carrots, and another group of 56 rats on the mixture of 85 per cent unpolished rice and 15 per cent dried baker's yeast, also supplemented with carrots. From 0.5 to 1.0 cc. of 2.5 per cent olive oil solution of dimethyl-amino-azobenzene, butter-yellow, was injected intraperitoneally once weekly during the entire experimental period. The results showed that among 6 rats in the group without yeast surviving 255 to 279 days, 3 were found with typical liver cancer, while for the corresponding period of survival none of the 10 rats in the yeast-fed group showed liver cancer. Rats in the first group were maintained upon an inadequate diet, especially lacking in vitamins. Rats in the second group were maintained upon a better balanced diet, although it was not an adequate diet.

From these experiments it seems that a certain factor or factors of diet may control the experimental development of liver cancer. Confirmation of the Japanese workers' findings and further studies on the role of diet in the development of cancer are imperative.

In the present study we used brewer's yeast (No. 2040, Fleischmann Laboratories) instead of baker's yeast, and fed dimethyl-amino-azobenzene instead of giving it by intraperitoneal injection.

The experimental group of 40 rats, Wistar and Sherman strains, was kept on the mixture of unpolished rice, 85 per cent, and dried yeast, 15 per cent, supplemented with a small slice of fresh carrot per rat daily.

The control group of 20 rats was maintained on unpolished rice without yeast, but supplied with fresh carrot.

Twenty cc. of 3 per cent butter-yellow solution in

olive oil were mixed well with 1,000 gm. of unpolished rice with or without yeast. Rats were allowed these mixtures *ad libitum*.

The results are presented in Tables VI and VII.

The data in Table VI show clearly that yeast feeding produced a very striking inhibition of liver cancer development. All of the 34 rats, with the exception of 2, examined between 104 and 284 days after the beginning of the experiment had smooth and practically normal livers. Rats Nos. 22 and 34, sacrificed on 223 and 284 days, respectively, had livers with slight granulation of the surface. Histologically they exhibited cirrhosis with adenomatous hyperplasia.

TABLE VI: EFFECT OF FEEDING WHOLE YEAST, 15 PER CENT, AND UNPOLISHED RICE ON THE PRODUCTION OF LIVER CANCER BY BUTTER-YELLOW

Rat no.	Sex	No. of days fed	Body weight (gm.) Initial	Maximum	Final	Liver findings *
1	♂	104	147	160	160	—
2	♂	104	134	174	174	—
3 †	♂	142	150	214	214	—
4	♀	150	153	190	177	—
5	♀	150	171	197	181	—
6	♂	150	154	163	150	—
7	♂	150	165	165	145	—
8	♀	177	163	165	165	—
9	♂	189	125	167	147	—
10	♂	189	120	164	138	—
11	♂	189	115	179	158	—
12	♂	200	145	168	158	—
13	♂	200	154	155	145	—
14	♂	200	120	148	118	—
15	♂	200	122	128	124	—
16	♂	200	121	150	140	—
17 †	♂	210	140	160	114	—
18	♂	221	131	176	176	—
19	♂	223	167	177	168	—
20	♀	223	164	180	166	—
21	♀	223	154	154	128	—
22	♀	223	133	133	132	±
23	♀	233	189	198	191	—
24	♂	233	204	242	204	—
25	♀	233	180	182	170	—
26	♂	233	190	190	178	—
27	♂	250	120	150	120	—
28	♂	250	138	154	135	—
29	♂	250	141	155	139	—
30	♂	250	130	159	145	—
31	♂	284	123	160	152	—
32	♂	284	121	169	163	—
33	♀	284	142	153	153	—
34	♂	284	139	175	145	±

* See Table III for explanation of symbols.
† Died.

In contrast, the animals fed the butter-yellow rice diet without yeast developed typical liver cancer in all cases in a comparatively shorter period of time. Rats Nos. 9, 11, and 12 of this group had large livers with large cancer nodules, and 2 of them also had metastases in the mesentery and omentum.

As may be seen in Table VI, about 90 per cent of the animals fed the butter-yellow rice diet with yeast gained weight and presented a healthy appearance throughout the experimental period. On the other hand, only 25 per cent of the rats of the control group receiving no yeast gained during the first 60 days, and thereafter their physical appearance was poor. All of the 12 rats of this group living 101 to 192 days died or were killed because of their terminal condition (Table VII).

Animals on the butter-yellow rice diet containing 15 per cent of yeast consumed from 7 to 9 gm. of the food daily. This amount is decidedly greater than the amount consumed by those fed on the butter-yellow rice diet without a supplement of yeast.

The inhibiting effect of yeast feeding upon the production of liver cancers is distinctly superior to that of the ether extract of yeast (Table V). In the latter case, the butter-yellow rice diet contained 0.25 per cent of yeast extract. From 10,000 gm. of dried yeast 165 gm. of ether extract were obtained. Therefore, 0.25 gm. of the ether extract is equivalent to 15 gm. of dried yeast. This suggests that the anticarcinogenic substance present in yeast is either not readily soluble in ether, or it is partially inactivated during the ether extraction.

TABLE VII: EFFECT OF UNPOLISHED RICE ALONE ON THE PRODUCTION OF LIVER CANCER BY BUTTER-YELLOW

Rat no.	Sex	No. of days fed	Body weight (gm.) Initial	Maximum	Final	Liver findings *
1	♂	101	135	135	122	+
2	♂	101	153	153	115	+
3	♂	104	134	134	70	+
4	♂	107	168	168	86	+
5	♀	125	140	140	93	+
6	♂	139	180	196	136	+
7	♂	156	145	145	138	+
8	♂	163	145	156	146	+
9	♂	167	155	157	134	++
10	♂	170	154	154	140	+
11	♂	188	169	169	168	++
12	♀	192	135	167	160	++

* See Table III for explanation of symbols.

EFFECT OF FEEDING PROTEIN

In other series of experiments, purified protein was added to the butter-yellow rice diet instead of yeast in order to determine whether or not an increased quantity of protein of known composition would inhibit the development of liver cancer.

Purified casein (18) was used in this series because it is known to contain all the amino acids essential to animal nutrition. It is especially rich in sulfur, containing amino acids such as cystine.

Twenty cc. of 3 per cent butter-yellow solution in olive oil were mixed well with 1,000 gm. of the mixture consisting of unpolished rice, 82 per cent, and purified casein, 18 per cent. As before, a small quantity of fresh carrot was given daily.

In this experiment we used 20 rats of the Wistar and Sherman strains. The results are presented in Table VIII.

The data in Table VIII show that the addition of milk protein had no inhibiting effect upon the production of liver cancer. A similar result was obtained by Nakahara, Mori, and Fujiwara (12), who used purified fish protein in their experiment.

TABLE VIII: EFFECT OF PROTEIN ON THE PRODUCTION OF LIVER CANCER BY BUTTER-YELLOW

Rat no.	Sex	No. of days fed	Body weight (gm.) Initial	Maximum	Final	Liver findings *
1	♂	100	174	184	117	—
2	♂	100	165	165	152	±
3	♀	100	147	147	126	±
4	♀	125	132	134	82	+
5	♀	134	77	108	108	—
6	♀	134	75	96	75	+
7	♀	159	65	125	80	+
8	♂	164	65	150	145	+
9	♂	164	160	183	130	++
10	♂	186	144	199	190	++
11	♂	198	133	165	155	++
12	♂	203	191	219	201	+
13	♂	210	149	174	125	++
14	♂	223	91	128	105	++
15	♂	234	149	156	156	++
16	♂	251	152	163	160	++
17	♂	284	173	204	180	+
18	♂	284	185	185	141	++
19	♂	284	125	167	158	++

* See Table III for explanation of symbols.

Animals on the protein-sufficient diet lived much longer than those maintained on a similar diet without an extra supply of protein. Many of these animals gained weight and appeared healthy throughout the experiment.

The livers of these animals grew larger and showed many large cancer nodules, mostly of the hepatoma type. Rats Nos. 9, 10, 11, 13, 14, 15, 16, 18, and 19 had very large livers and rats Nos. 9, 10, and 12 also had metastases in the mesentery and omentum.

EFFECT OF FEEDING 3 AND 6 PER CENT WHOLE YEAST

In the preceding experiments the failure of butter-yellow to produce liver cancers in rats fed on a diet of unpolished rice containing 15 per cent of yeast was clearly demonstrated.

In the following experiments an attempt was made to find out whether or not a smaller amount of yeast would have the same effect. In the present experiment 3 per cent and 6 per cent of yeast was added to the basal diets. Sugiura and Benedict (19) reported in an earlier paper that 3 per cent of yeast in a synthetic diet deficient only in vitamin B complex was sufficient to promote normal growth in young rats.

Fifty rats of the Sherman strain were used. One group of 10 rats was maintained on the mixture of unpolished rice, 94 per cent, and dried yeast, 6 per cent, and another group of 20 rats on the mixture of unpolished rice, 97 per cent, and dried yeast, 3 per cent. Twenty rats of the third group were kept on unpolished rice without yeast. All animals received butter-yellow in the proportion of 20 cc. of a 3 per cent solution per kilogram of food. In all 3 groups the rats received a small amount of fresh carrot daily.

After 150 days the experiment was discontinued; all rats then living were sacrificed and examined. The results obtained from the yeast feeding are presented in Tables IX and X. In every case, the diagnoses were confirmed by microscopic examination.

TABLE IX: EFFECT OF FEEDING WHOLE YEAST, 6 PER CENT, ON THE PRODUCTION OF LIVER CANCER BY BUTTER-YELLOW FOR 150 DAYS

Rat no.	Sex	Body weight (gm.) Initial	Maximum	Final	Liver findings *
1	♂	141	164	134	—
2	♂	136	171	146	—
3	♀	120	163	130	—
4	♀	118	145	103	—
5	♂	142	180	170	±
6	♂	132	132	100	±
7	♀	121	164	122	±
8	♂	130	160	143	+
9	♂	130	130	107	+
10	♀	125	135	116	+

* See Table III for explanation of symbols.

As may be seen in Table IX, the addition of 6 per cent yeast to unpolished rice had a distinct, although not complete, inhibiting effect on the progression of pathological changes leading to the development of liver cancer. Thus, among 10 rats, 4, or 40 per cent, had grossly and histologically normal livers; 3, or 30 per cent, cirrhotic livers; and 3, or 30 per cent, livers with a few small tumor nodules. The nodular livers contained cholangiomas. On the other hand, all of the 11 rats fed with butter-yellow rice diet without addition of yeast and examined on the 150th day, showed typical liver cancer.

The addition of 3 per cent yeast to the butter-yellow rice diet had a slight inhibiting effect upon the production of liver cancer. Among 20 rats, 6, or 30 per cent, showed no malignant change in the livers; 14, or 70 per cent, livers with numerous large and small tumor nodules. On histological examination these livers were

found to have cholangioma or hepatoma, or both. Rat No. 19 had metastases in the mesentery, and rat No. 20 had metastases in the lung, mesentery, and peritoneum.

The above results suggested the possibility that a vitamin-like substance or substances played a role in the inhibition of development of the liver cancers. Therefore, it is of interest to examine the riboflavin, vitamin B_2, content of the various food materials used in this study. Riboflavin estimations were made by the fluorometric method. The riboflavin content of the brewer's yeast employed was 70 γ/gm., yeast extract, 14.5 γ/gm., rice-bran extract, 0.5 γ/gm., brown rice, 0.5 γ/gm., and fresh carrot, 0.4 γ/gm.

TABLE X: EFFECT OF FEEDING WHOLE YEAST, 3 PER CENT, ON THE PRODUCTION OF LIVER CANCER BY BUTTER-YELLOW DURING 150 DAYS

Rat no.	Sex	Body weight (gm.) Initial	Maximum	Final	Liver findings *
1	♀	141	160	160	—
2	♂	121	180	164	—
3	♀	122	144	119	—
4	♂	144	153	140	±
5	♂	165	192	184	±
6	♂	122	167	125	±
7	♂	144	166	162	+
8	♀	136	150	137	+
9	♀	139	139	123	+
10	♂	118	118	118	+
11	♂	130	136	124	+
12	♂	118	119	117	+
13	♀	111	114	108	+
14	♀	127	136	118	+
15	♀	126	138	112	+
16	♂	120	165	155	++
17	♂	136	138	122	++
18	♂	146	160	131	++
19	♂	122	160	146	++
20	♂	140	164	124	++

* See Table III for explanation of symbols.

The brewer's yeast (No. 2040, Fleischmann Laboratories) contained vitamins B_1, 50-55 units/gm., B_2, 70 γ/gm., B_6, 60 γ/gm., H, 0.4 γ/gm., nicotinic acid, 90-340 γ/gm., pantothenic acid, 150-250 γ/gm., and ergosterol, 8.3 mgm./gm., but vitamins A, C, and E were absent.

SUMMARY AND CONCLUSIONS

1. Various factors which influence the production of liver cancer in rats by feeding p-dimethyl-amino-azobenzene, butter-yellow, have been investigated.

2. Under the conditions of the experiments only about 3 per cent of the rats died during the first month against 45 per cent reported by the Japanese workers.

3. The albino rats of the Sherman and Wistar strains were more susceptible to the carcinogenic action of butter-yellow than those of the Evans strain.

Of the 185 rats from the 3 strains which died or were sacrificed between 91 and 220 days after the beginning of the experiment 164, or 89 per cent, had liver cancer and the other 21, or 11 per cent, liver cirrhosis with or without bile duct adenomatosis. Of the 101 rats of the Wistar and Sherman strains which died or were sacrificed between 100 and 200 days 98, or 97 per cent, showed liver cancer and the other 3, or 3 per cent, had liver cirrhosis with or without adenomatous hyperplasia.

In the case of the Evans strain 19 out of 33 rats, or 58 per cent, living between 100 and 150 days, and 11 out of 13 rats, or 85 per cent, living between 151 and 180 days respectively had liver cancer, and all others, had liver cirrhosis with or without adenomatous hyperplasia. All of the 19 Evans rats surviving 181 to 220 days of butter-yellow feeding showed liver cancer.

4. The liver cancers were found to be of various histological types of hepatomas: trabecular or solid type; glandular or cholangioma type; and mixed type. Cirrhosis with adenomatous hyperplasia of bile ducts preceded these malignant tumors.

5. Daily ingestion of a small slice of fresh carrot has no noticeable effect on the production of liver cancer by butter-yellow, but it has an influence on the survival of the animal and consequently upon the type of liver cancer.

6. Rats fed butter-yellow and rice-bran extract (ether soluble) did not develop liver cancer during a period of 150 days. The inhibiting effect of the rice-bran extract upon experimental liver cancer appears to be transient because some of the rats finally developed the disease.

7. The development of liver cancer in rats fed butter-yellow is inhibited by the daily ingestion of a small amount of ether extract of yeast. The inhibition is less marked than that exerted by the rice-bran extract.

8. Butter-yellow did not produce liver cancer in rats fed on a diet of unpolished rice containing 15 per cent of brewer's yeast. All of the 34 rats examined between 104 and 284 days after the beginning of the experiment showed smooth and practically normal livers.

9. The inhibiting effect of yeast feeding upon the production of liver cancer was distinctly diminished when the basal diet of butter-yellow rice contained less than 15 per cent yeast. With the addition of 6 per cent yeast to the butter-yellow rice diet, 40 per cent of animals had normal livers; 30 per cent, cirrhotic livers; and 30 per cent, livers with a few tumor nodules when examined on the 150th day. With the addition of 3 per cent yeast to the basal diet, 30 per cent showed no malignant change in the livers; and 70 per cent,

livers with many large and small cancer nodules. On the other hand, all of the rats, 100 per cent, fed with butter-yellow rice diet without addition of yeast and examined on the 150th day, showed typical liver cancer.

10. The addition of purified protein, casein, to the butter-yellow rice diet had no inhibiting effect upon the production of liver cancer. Animals on the protein-sufficient diet lived much longer than those maintained on a similar diet without extra supply of protein. The livers of these animals grew larger and showed many large cancer nodules.

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Differences Between Malignant Blood Cells From Induced and Spontaneous Leukemias of Mice*

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Neoplasms may result from predominantly intrinsic genetic or extrinsic environmental factors or from a combination of the two. There is ample evidence of the significance of both in the genesis of neoplasms in animals (12).

Transmission experiments in animals of known genetic structure are suitable to characterize the genetic constitution of neoplastic cells. Such experiments might indicate a significant difference between neoplasms arising as a result of predominantly genetic factors and those caused by predominantly environmental factors.

It has been shown recently (18) that spontaneous leukemias arising in hybrid mice of which 1 parent was from a highly leukemic stock, Ak, and the other from a relatively nonleukemic stock, Rf, behave in transmission experiments like leukemias arising in the leukemic stock. They are transmissible to almost every member of the leukemic stock and to hybrids of the 1st filial generation, but not to members of the nonleukemic stock. This indicates that the leukemic cells arising in these hybrids resemble genetically the cells of the leukemic stock more closely than the cells of the hybrids in which the disease occurs.

It seemed desirable to determine whether malignant cells of leukemias induced in hybrids by carcinogens possess the genetic constitution of the hybrids or that of the ancestral leukemic stock. Neoplastic cells are regarded as mutants by several investigators (8) and by others as normal cells stimulated by ubiquitous agents or viruses to unrestrained multiplication (17). Should the neoplastic cells be mutants, their genetic constitution might differ from that of the cells from which they have arisen and from cells of the ancestors. It has been shown (3, 19) that the genetic constitution of different neoplasms arising spontaneously in the same host may differ, but this is not true for the spontaneous leukemias arising in Ak-Rf hybrids, the cells of which resemble each other and the Ak cells very closely.

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In order to obtain cells from induced neoplasms for comparison with those from spontaneous neoplasms, mice of known genetic constitution were treated with carcinogenic chemicals. These experiments are described in the first part of this paper. A genetic analysis of malignant blood cells of induced leukemias made by means of transmission experiments is described in the second part. Observations with carcinoma and sarcoma cells will be presented elsewhere.

Incidence of spontaneous neoplasms among the experimental animals.—Mice of stocks Ak, Rf, and of hybrid combinations were used. Stocks Ak and Rf have been inbred for more than 20 generations; their origin has been described by Furth, Seibold, and Rathbone (10).

A recent preliminary analysis of the incidence of spontaneous leukemia and tumors among the purebred and hybrid mice, to be described more fully elsewhere (11), is as follows:

Stock of mice	Number of mice	Lymphoid and myeloid leukemia, per cent	Lung tumors, per cent
Ak	214	74	0
Rf	167	2	26
F1 (a) [Ak♀xRf♂]	180	22	12
F1 (b) [Rf♀xAk♂]	151	12	18
F2 [F1(a)xF1(a)]	238	12	12
F2 [F1(b)xF1(b)]	213	9	19

Most of the leukemias in stock Ak are of the lymphoid type; a few are myeloid. Spontaneous leukemia is rare in stock Rf and the type is usually monocytic or myeloid. Other types of tumors are almost nonexistent among mice of stock Ak. Lung tumors are frequent among Rf mice, but other neoplasms are rare (2).

Transmission of leukemia.—Transmission of leukemia was attempted by injecting in the tail vein of mice 0.1 cc. of a suspension of cells from spleen or spleen and lymph nodes from a mouse with leukemia, as described previously (10).

Experimental Production of Neoplasms

The results of 14 experiments made during a period of 3 years are summarized in Table I. Nearly every mouse receiving methylcholanthrene or benzpyrene developed 1 or several neoplasms. The subcutaneous injection of the chemical resulted in the development of sarcoma or carcinoma at the site of injection and in an increased incidence of lung tumors. The data indicate that the incidence of leukemia was increased

TABLE I: NEOPLASMS IN MICE PRODUCED BY CARCINOGENS

Experiment no.	Stock of mice	Carcinogen: Chemicals*	Carcinogen: Route	Carcinogen: Dose*	Mice: No. in exp.	Mice: Age at start (months)	No. with neoplasms: Subcutaneous	No. with neoplasms: Cutaneous	No. with neoplasms: Lung	No. with neoplasms: Leukemia	No. with no neoplasms	Attempted transmission: Leukemia: No. −	Attempted transmission: Leukemia: No. +	Attempted transmission: Sarcoma and carcinoma: No. −	Attempted transmission: Sarcoma and carcinoma: No. +
1	Ak	M	subcutaneous	8	8	2	8	0	7	0 (1?)	0	..	..	0	3
2	Ak	M	percutaneous	34	20	1¼	0	2	4 ‡	10 (+ 1?)	7	1	2	..	..
3	Ak	M	percutaneous	24	13	¾	0	1	1 ‡	9	2	1	2	..	..
4	Rf	B	subcutaneous	17.5	3	10	2	1	0	1 (+ 2?)	0	..	..	1	2
5	Rf	M	percutaneous	16	10	1	0	10	6 ‡	4 (+ 1?)	0	0	2	..	..
6	Rf	M	subcutaneous	8	12	2	9	0	10	1 (+ 1?)	2	..	..	3	1
7	Rf	M	percutaneous	24-38	22	1	0	16	22	7 (+ 2?)	..	2	2	..	..
8	F1	B and M	subcutaneous	17.5	11	1 to 4½	8 (+ 1?)	1	0	4 (+ 1?)	0	..	..	4	2
9	F1	M	subcutaneous	8	23	2	21	0	18	5 (+ 1?)	0	1	1	2	1
10	F1	M	percutaneous	39	20	1½	0	13	12 ‡	13 (+ 1?)	2	..	..	..	..
11	F1	M	percutaneous	22	17	¾	0	0	14 ‡	16	1	1	3	..	..
12	F2	B and M	subcutaneous	17.5	9	2	1	2	0	7	0	..	..	1	1
13	F2	M	percutaneous	8	16	1	12	2	15	4 (+ 1?)	0	0	2	0	2
14	F3	B	subcutaneous	17.5	5	2	4	0	2	1	0	..	..	0	3

* Chemicals used: M = methylcholanthrene, B = 1,2-benzpyrene. Dose refers to the amount in mgm. injected subcutaneously, or to the number of cutaneous applications.
‡ The number of lung tumors produced in these experiments is probably more than the number stated. Lungs from 4 mice in the 1st and 5th experiments, from 3 mice in the 2nd and 10th experiments, and from 1 mouse in the 11th experiment could not be examined.

by the subcutaneous injection of carcinogens, in the F1 and F2 hybrids.

It is also evident that the incidence of leukemia was greatly increased by the percutaneous application of methylcholanthrene in the relatively nonleukemic Rf mice, and in F1 and F2 hybrids, while there were fewer cases of leukemia among the painted mice of the leukemic stock Ak than among the controls:

Stock of mice	Ak	Rf	F1	F2
Number of mice painted with methylcholanthrene	33	32	37	16
Percentage of leukemia	58	34	78	25
Number of control mice	214	167	331	451
Percentage of spontaneous leukemia	70	4	21	12

In order to evaluate these data it is desirable to record the incidence of both spontaneous and induced leukemias according to the age of the mice. Table II shows that the peak occurrence of spontaneous leukemia in mice of Ak stock was at from 8 to 9 months of age, whereas the treated mice died of this disease at the age of from 4 to 5 months. All painted mice in the experiment were dead before the age of 10 months. It is evident that the chemical hastened the development of leukemia, and that a consideration of the incidence of the disease only, without considering the age of the experimental animals, is misleading.

Table II shows that painting mice of the relatively nonleukemic stock Rf with methylcholanthrene hastened the development of leukemia and greatly increased its incidence. Among the painted mice of this stock leukemia occurred at the age of from 4 to 5 months, while most spontaneous leukemias in this stock occurred after the age of 13 months. Most spontaneous leukemias arising in this stock were monocytic or myeloid; all induced leukemias were lymphoid.

In the 1st filial generation of hybrids between the leukemic stock Ak and the nonleukemic stock Rf, spontaneous leukemia occurred at a relatively late age (Table II). The incidence of leukemia in the F1 hybrids was approximately 1/3 that observed in the leukemic stock, and most cases of leukemia occurred after 14 months of age in comparison with a peak incidence of from 8 to 9 months in the leukemic stock. All but 1 painted mouse of the F1 hybrids living longer than 6 months died of leukemia. Subcutaneous injection of carcinogens also increased the incidence of disease and hastened its development.

The behavior of the F2 hybrids is similar to that of the F1 hybrids. Although the number of experimental mice is small, the data are sufficient to indicate that both subcutaneous and percutaneous applications of methylcholanthrene increased the incidence of leukemia and hastened its occurrence.

The data of Table I also suggest that leukemia is more readily induced in mice of approximately 3 weeks of age than in those of from 5 to 7 weeks of age. In Experiment 11, 16 of 17 painted mice, 3 weeks of age, had leukemia; whereas in Experiment 10, only 13 of 20 painted mice, 7 weeks of age, had this disease, even though the older mice received 39 percutaneous applications of the chemical while the younger mice received only 22. In Experiment 3, leukemia developed in 9 of 13 mice, 3 weeks of age, receiving 24 percutaneous applications of methylcholanthrene, as compared with 10 of 20 mice, 6 weeks of age, that received 34 applications of the chemical (Experiment 2). The number of mice in the 2 experimental groups is small and their age difference is not great. The relation of age to the response of percutaneous and subcutaneous application of methylcholanthrene deserves further study.

Neoplasms other than leukemia.—It is well known that painting of mice by carcinogens increases the incidence of lung tumors (1, 13, 16). Our data (Table I) do not give an accurate picture of the incidence of this neoplasm because many of the animals were killed or died with leukemia or with cutaneous or subcutaneous tumors at a relatively early age. Nevertheless, they are sufficient to indicate that the incidence of lung tumors was greatly enhanced both by cutaneous and subcutaneous application of the carcinogen in all stocks studied. This neoplasm is almost absent in our stock Ak and has not been seen in mice of stock Rf before the age of approximately 8 months. In a study on the pathogenesis of lung tumors induced by methylcholanthrene every mouse of F2 hybrids surviving 5 months of age had pulmonary tumors. The number of lung tumors in most mice of this series exceeded 100 (5).

The data on the incidence of cutaneous tumors (Table I) is likewise inaccurate. The incidence of papillomas in the earlier series was not faithfully recorded. In many mice, 1 or several papillomas changed into carcinoma, but the character of the cutaneous tumors has not been investigated.

TABLE II: THE OCCURRENCE OF SPONTANEOUS AND INDUCED LEUKEMIA IN RELATION TO AGE

Mice	Age in months: 3 or less	4	5	6	7	8	9	10	11	12	13	14	Over 14
Ak Untreated													
Leukemic	..	..	..	**2**	**10**	**30**	**30**	**22**	**1**	**10**	**6**	**4**	**5**
Nonleukemic	7	3	4	4	2	8	8	5	9	6	10	4	8
Treated													
Percutaneous													
Leukemic	**1**	**8**	**9**	**1**	..	..	..	..	..	..	..	..	..
Nonleukemic	..	3	6	3	1	..	1	..	..	..	..	..	..
Rf Untreated													
Leukemic	..	..	..	..	..	**1**	..	..	..	..	**1**	**1**	**3**
Nonleukemic	2	3	4	6	4	10	7	4	15	19	12	24	79
Treated													
Subcutaneous													
Leukemic	..	..	..	..	..	..	..	**1**	..	..	..	**1**	..
Nonleukemic	..	..	2	..	..	4	..	2	..	1	1	2	1
*Percutaneous													
Leukemic	..	**4**	**6**	**1**	..	..	..	..	..	..	..	..	..
Nonleukemic	..	2	1	3	..	..	..	..	..	..	..	..	..
F1 Untreated													
Leukemic	..	..	..	..	**2**	**6**	**3**	**5**	**1**	**0**	**5**	**4**	**43**
Nonleukemic	7	2	1	6	1	2	7	16	12	17	11	26	183
Treated													
Subcutaneous													
Leukemic	**1**	**1**	..	**6**	**1**	..	..	..	..	..	..	..	..
Nonleukemic	..	1	3	12	6	..	2	..	1	..	..	..	..
Percutaneous													
Leukemic	**1**	**9**	**5**	**1**	**10**	**3**	..	..	..	..	..	..	..
Nonleukemic	..	..	5	2	..	1	..	..	..	..	..	..	..
F2 Untreated													
Leukemic	..	..	**1**	..	..	**6**	**4**	**5**	**4**	**6**	**6**	**7**	**17**
Nonleukemic	6	3	2	5	3	8	9	8	24	26	30	33	255
Treated													
Subcutaneous													
Leukemic	..	**2**	**5**	..	..	..	..	..	..	..	..	..	..
Nonleukemic	..	..	1	1	..	..	..	..	..	..	..	..	..
Percutaneous													
Leukemic	..	**2**	**2**	..	..	..	..	..	..	..	..	..	..
Nonleukemic	..	6	3	2	1	..	..	..	..	..	..	..	..

* These data are incomplete; mice killed at various intervals in a study of the pathogenesis of induced leukemia are omitted.

Transmission of Induced Leukemias

The data summarized in Tables I and II indicate that methylcholanthrene increases the incidence of leukemia and hastens its development. The question arises whether this effect is due to a lowering of the host's resistance to neoplastic cells which are present in a latent state in apparently healthy animals, as suggested by Cohnheim (7) and recently by Fischer (9), or to a malignant transformation of normal cells. The stocks employed, Ak and Rf, are unusually suitable for the investigation of this problem because the spontaneously in this stock and could not be transmitted to Rf mice. Three of these 4 strains that were tried were passed successfully to F1 hybrids. One strain was successfully transmitted to Rf mice in 2 of 9 experiments.

Leukemias induced in stock Rf.—All 3 strains were readily transmissible to Rf mice and could also be grafted on mice of the Ak stock; 2 with ease, and 1 with difficulty. The number of successful inoculations was greatest in the Rf stock, as great or almost as great in the F1 stock, and moderate or much less in

Table III: Transmissibility of Spontaneous Leukemia *

Leukemia occurring in	Grafted on				
	Stock Ak	Stock Rf	F1 hybrid	F2 hybrid	F3 hybrid
Stock Ak	Excellent	Failed	Excellent	Good	Fair
Stock Rf	Failed	Excellent	Excellent	Fair	Fair
F1 hybrids	Excellent	Failed	Excellent	Variable	Fair
F2 hybrids	Excellent	Failed	Excellent	Fair	Fair
F3 hybrids	Excellent	Failed	Excellent	Fair	Fair

* Designation of success of inoculation: Excellent = approximately 100 per cent; Good = 75 per cent; Fair = 50 per cent; Poor = 25 per cent; Failed = 0 per cent.

Table IV: Transmission Experiments of Leukemias Induced by Carcinogens

Leukemia		Mice receiving injection of leukemic cells															
		Ak				Rf				F1				F2			
		No. of mice				No. of mice				No. of mice				No. of mice			
Stock of origin	Strain	No. of experiments	Injected	+	Per cent +	No. of experiments	Injected	+	Per cent +	No. of experiments	Injected	+	Per cent +	No. of experiments	Injected	+	Per cent +
Ak	Ak 946	7	39	28	72	9	62	6	10	4	22	16	73	2	12	1	8
	Ak 966	4	25	15	60	3	16	0	0	..	..	..	..	..	..	..	..
	Ak 970	11	63	59	94	6	36	0	0	3	13	12	92	1	6	3	50
	Ak 1030	5	27	26	96	4	19	0	0	2	9	7	78	..	..	..	..
Rf	Rff 25	8	47	40	85	4	17	17	100	3	16	16	100	..	..	..	..
	Rff 27	6	32	16	50	2	13	12	92	6	30	26	87	3	17	15	88
	Rff 212	8	47	2	4	9	46	46	100	3	12	12	100	..	..	..	..
F1	Ma 131	7	35	26	74	3	17	4	23	12	58	46	79	2	11	7	64
	Ma 529	5	23	4	17	3	16	0	0	5	26	18	69	..	..	..	..
	Ma 531	5	26	0	0	5	29	0	0	5	24	19	79	..	..	..	..
	Ma 562	3	16	0	0	3	16	0	0	4	18	16	89	..	..	..	..
F2	Ma 61	10	56	42	75	4	29	5	17	13	72	55	76	3	20	13	65
	Ma 62	5	27	15	56	5	35	0	0	12	70	43	61	3	23	9	39

spontaneous leukemias arising in their hybrids resemble in transmission experiments the leukemias arising in the ancestral leukemic stock (Table III) (12). Should carcinogens bring about a malignant transformation of normal cells, it is likely that in hybrids the neoplastic cells will resemble hybrid cells more closely than those of either parental stock. Of 83 leukemias induced by carcinogens, attempts were made to transmit 20. Fourteen of these attempts were successful and the results of 13 tested genetically are summarized in Table IV.

Leukemias induced in stock Ak.—Three of 4 strains of transmissible leukemia induced in Ak mice behave in transmission experiments like those originating the Ak stock. Two of the transmissible strains induced in this stock, Rff 25 and Rff 27, were likewise passed to mice of F3 generation. In 5 experiments made with strain Rff 25, 21 of 29 injected mice, 72 per cent, developed leukemia. In 1 experiment made with strain Rff 27, 2 of 8 mice had leukemia.

Leukemias induced in F1 hybrids.—Two of the 4 transmissible strains could be readily grafted on F1 hybrids but not to mice of either parental stock. A 3rd strain, on the contrary, was nonspecific and could be grafted to members of both parental stocks, although the percentage of successful inoculation was highest in F1 hybrids. The 4th strain could be transmitted to an occasional mouse of the ancestral leu

kemic stock, Ak, not at all to mice of the ancestral nonleukemic stock, and fairly well to F1 hybrids. Thus 3 of the 4 transmissible leukemias produced in F1 hybrids differed genetically from each other as well as from the spontaneous leukemias arising in F1 hybrids.

Leukemias induced in F2 hybrids.—One of the 2 strains behaved like the spontaneous leukemias arising in this stock in that it could be grafted on F2 and F1 hybrids and on Ak mice, but not on Rf mice. The percentage of successful inoculations was only moderate. The 2nd transmissible strain was nonspecific and could be transplanted to mice of all stocks tested.

Discussion

These studies indicate that leukemia can be readily produced by carcinogens in mice of stocks Ak, Rf, and in their hybrids. Morton and Mider (14, 15) have already shown that painting of mice of a susceptible stock with methylcholanthrene over long periods of time hastens the occurrence of leukemia. Brues and Marble (6) found that painting mice of Bagg's albino stock with a carcinogenic tar increased the incidence of lymphoblastoma and lymphatic leukemia from 2 per cent to 50 per cent, but similar treatment of C57 mice failed to produce lymphomatosis. The experiments described here indicate that the subcutaneous injection of this chemical is likewise productive of leukemia, although painting is the more effective procedure in producing the disease.

The leukemias induced by carcinogens are about as readily transmissible to mice of the stocks of origin as the leukemias arising spontaneously. Previous studies have shown that the leukemias arising spontaneously in the highly leukemic stock Ak cannot be transmitted to Rf mice. The present experiments show that this is also true for 3 of 4 induced leukemias. The 4th strain could also be grafted on Rf mice, though with difficulty. The spontaneous leukemia arising in the Rf stock that was analyzed genetically (18) could be readily grafted on Rf mice but not at all on Ak mice. All 3 leukemias induced in this stock, on the contrary, could be grafted on Ak mice.

The critical experiment in establishing a difference between spontaneous and induced leukemias involves a study of this disease occurring in hybrids. All spontaneous leukemias observed in F1 and F2 hybrids that were transmitted resembled the Ak leukemias and could be grafted on Ak mice as well as on hybrid mice, but not at all on Rf mice. In contrast to this, none of the 4 leukemias induced in F1 hybrids was of the Ak type. One was similar to the Ak leukemias in that it could be grafted on an occasional mouse of this stock, but it could be passed more readily to F1 mice. Two of the strains seemed to require genes from both parental stocks for successful transplantation, for they could be grafted only on hybrid mice and not on mice of either parental stock. The 4th strain, on the contrary, was nonspecific and could be grafted on mice of all stocks tested, although the percentage of successful inoculations was lower in Rf mice than in Ak mice. One of the 2 strains of leukemias induced in F2 hybrids was similar to the latter, while another strain was of the Ak type.

Spontaneous neoplasms arising in hybrids of known genetic constitution have thus far been given little consideration (18). Moreover, most of the spontaneous neoplasms studied from the genetic standpoint were breast tumors, which, in the light of recent investigations (4), can no longer be regarded as true hereditary neoplasms.

Experiments are in progress to compare the character of spontaneous and induced neoplasms other than leukemias. The data thus far accumulated indicate that the breast tumors induced by the extrachromosomal "nursing factor" of Little and Bittner (4) in the Ak stock resemble in transmission experiments the neoplasms of stock Ak and not those of the stock C3H from which the nursing factor is derived. Spontaneous neoplasms other than leukemia are rare in the stocks studied, and it is possible that the few that were observed are not truly hereditary neoplasms. Nevertheless, we have undertaken a study of the genetic constitution of both spontaneous and induced tumors in these stocks. The tumors induced by methylcholanthrene in Ak, Rf, and hybrid mice differed among themselves in transmission experiments. Some tumors induced in Ak or Rf mice were transmissible to mice of the stock in which they arose, and to hybrids, but not to mice of unrelated stocks; others could be grafted on both Ak and Rf mice and their hybrids. Tumors induced in the hybrids could be passed to neither, to one, or to both, of the parental stocks, as well as to mice of the hybrid combination in which they arose.

Summary

Leukemia was produced by the administration of carcinogenic chemicals in mice of a leukemic stock, Ak, in mice of a stock, Rf, in which spontaneous leukemia is rare, and in hybrids of these stocks.

The genetic structure of neoplastic blood cells from induced leukemias was tested in transmission experiments using purebred and hybrid mice of these stocks. Previous experiments have shown that the spontaneous leukemias arising in these hybrids are, in transmission experiments, similar to the spontaneous leukemias arising in leukemic stock. The present experiments have shown that the induced leukemias differ among themselves. Only 1 of 6 transmissible leukemias induced in

hybrids resembled closely those arising spontaneously. Two were nonspecific and could be grafted on both parental leukemic and nonleukemic stock, and 2, on the contrary, could be readily grafted on hybrids but on neither parental stock.

Conclusion

Leukemic cells from spontaneous and induced leukemias may differ genetically as indicated by transmission experiments. This difference may be explained by assuming that the cells of the induced leukemias are mutants.

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Immunity Induced Against the Brown-Pearce Carcinoma

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The first investigators (4, 5, 13) of the Brown-Pearce carcinoma, a highly malignant epithelioma arising in a rabbit's testicle, noted that this transplantable tumor belongs to the group, the members of which as they grow tend to immunize the host against subsequent inoculations of similar material. If a transplanted tumor grows temporarily and then regresses, the animal is rendered refractory to subsequent implantations of the carcinoma. The mechanism of this immunity has been studied by Besredka and his collaborators (1-3), who could not demonstrate the existence of either humoral or tissue antibodies. Their method of immunization consisted in the implantation of an intracutaneous tumor which in 94 per cent of the cases retrogressed, usually within a month, leaving the animal solidly immune against subsequent intratesticular inoculations of tumor material. The control animals invariably developed fatal metastasizing lesions. The efficacy and practicability of this method of immunization have been investigated by several workers (9, 10, 14, 15) with conflicting results. Since nonspecific factors are known to influence the percentage of positive results in any series of attempted implantations of this tumor (6-8), it seemed advisable to repeat Besredka's fundamental experiments over the course of an entire year, employing a standardized technic.

Experimental Studies

Technical details.—The rabbits, obtained from 5 local dealers, were in general a hybrid lot, consisting of New Zealand whites, Belted Dutch, and English Butterflies, together with a number of frank crosses. There appeared to be no great variation in the proportion of these types received throughout the year, nor was any significant difference observed in the percentage of tumor takes among the various breeds. For the most part the animals were housed in single cages under similar conditions of food, care, etc. During the year a number of rabbits were lost through nonspecific causes. One series of deaths occurred in the summer, when an epidemic of diarrhea persisted intermittently throughout the hot weeks, and a smaller one in the late winter, when a few animals were lost with snuffles and a hemorrhagic type of pneumonia.

Animals bearing suitable growths were bled by cardiac puncture and killed with ether. Both testicular material and metastatic nodes were used for inoculation; often the 2 types were mixed. No significant difference was observed between them. The tumor was removed with aseptic precautions, and any necrotic tissue was trimmed away. The material was then finely minced with scissors, approximately 3 to 4 times its weight of physiological saline or broth was added, and the mass was then ground up in a mortar with the addition of a small amount of sterile Pyrex glass. The resulting emulsion was permitted to stand in a tall 50 cc. centrifuge tube for 5 minutes in order to permit the glass and coarser particles to settle. Only the upper half of the suspension was used, portions of which were injected by means of an 18 or 20 gauge needle. The standard intratesticular or intradermal dose was 0.4 to 0.5 cc. The animals were inspected at regular weekly intervals; and in many cases oftener. All animals were autopsied, and where any doubt existed as to the nature of a suspected lesion microscopic sections were made.

Testicular inoculation.—During the course of the year 107 animals were injected intratesticularly with freshly prepared tumor material with the following results:

Course	Number	Percentage
Take in 1 or both testicles, with or without metastases, at autopsy	77	72
Clinical take in 1 or both testicles followed by spontaneous regression	7	6.5
No clinical take. Testicles negative at autopsy	23	21.5
Total	107	100

Thirteen out of 23 animals which proved refractory to the first intratesticular inoculation received a second injection between 6 and 9 weeks later, with the following results:

Course following second inoculation	Number	Percentage
Take in 1 or both testicles, with or without metastases, at autopsy	3	23
Clinical takes in 1 or both testes, with subsequent regression	0	0
Negative clinically and pathologically	10	77
Total	13	100

Approximately 1 rabbit out of 5 appeared to be naturally refractory to intratesticular inoculations of the tumor. Some of the testicular growths regressed spontaneously; this number might have been larger had it not been necessary to sacrifice some of the animals within a month of the date of inoculation in order to obtain fresh material for further tumor transplants.

Intradermal inoculation.—During the course of the year 50 animals received intradermal inoculations of tumor material, with the following results:

Course	Number	Percentage
Positive takes: metastases, death	3	6
Positive takes: regression	37	74
Positive takes: death of animal from intercurrent infection before fate of tumor could be determined	5	10
Failure to take	5	10
Total	50	100

Of the 5 animals which proved refractory to the primary intradermal inoculation, 2 were subsequently re-injected in the skin. In both cases typical skin takes occurred, which in the course of 4 weeks regressed in the usual manner. These 2 animals were added to the 37 which had developed skin tumors after their 1st injections, and in which the growth subsequently regressed. Four animals of this series were not available for later intratesticular inoculation, leaving a total of 35 animals for the test series. In the majority of cases the skin tumors had regressed completely previous to the testicular inoculation; in a few regression was not yet complete.

Intratesticular inoculation subsequent to the development and regression (complete or partial) of skin tumors.—The results in 35 animals were as follows:

Course	Number	Percentage
Positive takes, followed by regression	1	2.8
Questionable takes: pathological report uncertain	1	2.8
Death of intercurrent diseases within 3 days of intratesticular inoculation; no autopsy.	1	2.8
No take	32	91.6
Total	35	100

Of the 32 rabbits refractory to intratesticular inoculation, 21 received a 2nd injection of tumor material into the testes, with results as follows:

Course	Number	Percentage
Positive take in one testicle, subsequent regression	1	4.8
No take	20	95.2
Total	21	100

The viability of tumor material injected intradermally or intratesticularly was demonstrated in each instance by the simultaneous intratesticular inoculation of the same material into normal animals. In every case at least 1 of the control animals developed a typical testicular lesion. Complete or partial regression of a skin tumor was followed by the development of a refractory state against subsequent intratesticular inoculation of tumor material in over 90 per cent of the animals tested.

Intradermal inoculation: Time factor.—In order to investigate the relation of the state of the skin growths to the fate of tumor material subsequently injected intratesticularly, the following experiment was devised. Two rabbits were inoculated intradermally in 5 places with freshly secured nonnecrotic tumor material. Two more rabbits were inoculated 1 week later, and the process was repeated until, at the end of the 5th week, 10 animals were available with tumors in various stages of progression and regression. In 2 instances the tumors had appeared and completely regressed, in 3 the skin tumors had reached their maximum size; in one of these an axillary node could be palpated, subsequently regressing. In one animal the skin tumors had coalesced to give an *en cuirasse* effect with several palpable axillary nodes; in another the skin tumors were rapidly growing, while in the remaining 3 intracutaneous nodules had not yet appeared, although they did so later. At this time all 10 animals and 2 normal ones, which served as controls, received bilateral intratesticular inoculations. The results obtained are recorded in Table I.

It appeared that the simultaneous intradermal and intratesticular inoculation of tumor material did not affect the subsequent development of a testicular lesion; in contrast to this the intradermal inoculation of malignant cells 1 week or more prior to intratesticular inoculation apparently conferred a state of solid immunity against subsequent infections, no matter what the state of the skin tumor was.

Nonspecific tissues.—To investigate the possibility that nonspecific tissue factors might induce a state of resistance to the growth of tumor cells, rabbit embryo skin was selected as a possible immunizing agent, since this has been reported as being the most effective in this respect. Cesarean section on a pregnant rabbit was performed and rabbit embryo skin procured under sterile conditions. This was finely macerated with scissors and ground in a mortar; approximately 3 to 4 times its volume of saline was added, and the resulting emulsion was injected intradermally in 0.5 cc. amounts in 5 places on the shaved flanks of each of 12 rabbits. In 3 animals small pea-sized nodules appeared and persisted for 3 or 4 days. In the others no more than a transient erythema at the site of inoculation was observed. Three weeks later the course was repeated with similar results, and 12 days after this each animal

received a double intratesticular inoculation of tumor material, a control series of normal animals being similarly inoculated at the same time. The results were as follows:

Animals Treated with Inoculations of Fetal Skin

Course	Number
Takes proven at autopsy	6
Takes (clinical) followed by regression	0
Death on 3rd day after intratesticular inoculation. No autopsy	1
No take	5
	12

Untreated Animals

Course	Number
Takes proven at autopsy	5
Takes (clinical) followed by regression	1
No take	0
Total	6

Nonspecific tissue thus appeared to have an immunizing effect which, however, was much less marked than that exerted by tumor cells themselves.

Pathological findings.—Microscopic sections were made of several series of skin tumors in progressive and regressive stages, but nothing specific was noted as to the mechanism of regression. In the earliest stages, 4 days to 1 week, the injected tumor cells appeared much disordered, islands of apparently healthy, multinucleated, infiltrating cells being separated from each other by strands of connective tissue and areas of necrotic tumor cells. Then ensued a period of rapid growth, the tumor forming a solid sheet of large cells with vesicular nuclei and many mitotic figures. The infiltrating cells forced their way deep into the corium, and in some cases reached the fatty subcutaneous tissues and even the muscle bundles themselves. Very little reaction at the periphery of the tumor was noted at this time. The first evidence of necrosis always appeared near the center of the growth, usually by the 10th or 12th day. It was often accompanied by hemorrhage and was as a rule fairly well advanced before peripheral changes were noted. Henceforth, the pathological process appeared to follow 1 of 2 courses: (a) The epidermis overlying the tumor ruptured permitting the development of secondary infection in the form of a localized abscess with subsequent sloughing out of the necrotic tumor core, followed by fibrosis and the laying down of new connective tissue elements which obliterated the site of the original inoculation. (b) Infection did not occur. The degenerative process was initiated by the gradual development of an inflammatory reaction about the periphery of the tumor, characterized by round cell infiltration followed by the development of a capsule composed of dense thickly interwoven strands of connective tissue. Central necrosis of the tumor continued rapidly but patches of malignant cells remained healthy in the periphery of the mass, and in islands surrounding more deeply situated blood vessels. In

TABLE I: INTRADERMAL INOCULATION: TIME FACTOR

Number of rabbit	Date of intradermal inoculation	Result of intradermal inoculation	State of intradermal tumor at time of intratesticular inoculation	Date of testicular inoculation	Result of testicular inoculation
360	Jan. 23, 1940	5 skin tumors, subsequent regression	Maximum size	Feb. 19, 1940	No take
362	Jan. 23, 1940	5 skin tumors, subsequent regression	Maximum size	Feb. 19, 1940	No take
353	Jan. 29, 1940	5 skin tumors, subsequent regression	Maximum size; 1 axillary node	Feb. 19, 1940	No take
354	Jan. 29, 1940	5 skin tumors, metastases, death	Coalesced nodules; axillary nodules	Feb. 19, 1940	No take
405	Feb. 5, 1940	5 skin tumors, subsequent regression	Tumors completely regressed	Feb. 19, 1940	No take
406	Feb. 5, 1940	2 skin tumors, subsequent regression	Tumors completely regressed	Feb. 19, 1940	No take
422	Feb. 12, 1940	5 skin tumors, subsequent regression	Tumors growing	Feb. 19, 1940	No take
423	Feb. 12, 1940	3 skin tumors, subsequent regression	Tumors not yet appeared	Feb. 19, 1940	No take
430	Feb. 19, 1940	3 skin tumors, subsequent regression	Simultaneous inoculation	Feb. 19, 1940	Take in left testicle
432	Feb. 19, 1940	No skin tumors developed	Simultaneous inoculation	Feb. 19, 1940	Take in right testicle
431				Feb. 19, 1940	Take in right testicle
433				Feb. 19, 1940	Take in right testicle, subsequent regression

some cases it was impossible to be sure whether a given cell was a degenerated proliferative one with a pyknotic nucleus, or an infiltrating round cell of inflammatory origin. Small hemorrhagic areas were usually noted. The islands of healthy tumor cells showing numerous mitotic figures persisted for some time, but ultimately they too were choked off by inflammatory cells and granulation tissue, and at the end of a month nothing was left but a fibrous scar which microscopically showed large areas of lipoid laden cells and of hyaline degeneration. The peripheral process appeared to be entirely independent of the central necrosis, which occurred even in tumors which exhibited rapid marginal extension.

Humoral antibodies.—Several experiments were carried out to confirm the absence of demonstrable humoral antibodies as reported by Besredka (1-3). "Immune" serum; *i.e.,* serum from an animal whose skin tumors had regressed and which had proven refractory to subsequent intratesticular inoculation, was injected in and around both intradermal and intratesticular growths without producing any evidence that the subsequent course of the tumors was affected in the slightest degree. Clamping off the base of the tumor or of the scrotal sac in accordance with Lumsden's (12) suggestion was equally ineffective. As a final experiment tumor material was collected aseptically, divided into 3 parts and emulsions made, using the 3 following fluids as bases for the respective portions: immune serum, normal serum, and saline. Each emulsion was then placed in a 37° C. incubator for 120 minutes with frequent agitation before being injected into the testicles of normal rabbits. The results were as follows:

Immune Serum

Course	Number
Takes proven at autopsy	3
Takes (clinical) with subsequent regression	1
No takes	4
Total	8

Normal Serum

Course	Number
Takes proven at autopsy	2
Takes (clinical) with subsequent regression	1
No takes	3
Total	6

Saline

Course	Number
Takes proven at autopsy	2
Takes (clinical) with subsequent regression	0
No takes	4
Total	6

Eight of the 11 rabbits refractory to the 1st inoculation were given a 2nd inoculation 6 weeks later with tumor material prepared in the standard way using saline as a diluent and no period of incubation. None of these animals developed tumors while of 2 normal controls inoculated simultaneously 1 developed bilateral lesions with widespread metastases.

So far as these experiments went there was no evidence to suggest the existence of humoral antibodies against the tumor cells. With so few animals available, however, it is obviously impossible to draw definite conclusions.

Discussion

These experiments, carried out over the course of a year with a reasonably standardized technic, brought out several points. 1. Intratesticular inoculation of tumor material gave over 70 per cent takes. A number of testicular growths regressed spontaneously. 2. Intradermal inoculation of tumor material gave an apparent incidence of 90 per cent takes. The word "apparent" is used advisedly since in the case of some of the smaller and more transient tumors it was impossible to be sure that actual but temporary multiplication and proliferation of the tumor cells had taken place rather than a mere foreign body reaction consequent upon or subsequent to the death of the injected neoplastic cells. The great majority of these skin tumors regressed spontaneously. 3. Immunity following this regression ran over 90 per cent of the animals tested. 4. Complete regression of the skin tumor was not necessary for the development of a generalized immunity; skin tumors in all stages of progression and regression seemed equally effective in producing it. On the other hand, simultaneous intradermal and intratesticular inoculation did not appear to affect the normal course of the latter. 5. A certain degree of immunity could be obtained by the injection of homologous normal tissues; *e.g.,* fetal rabbit skin. 6. Gross and microscopic study of skin tumors as they regressed yielded no clues as to the nature of the factors involved in this process of regression. 7. In a small and hence statistically nonsignificant series of experiments no evidence suggesting the existence of humoral antibodies was observed.

This repetition of Besredka's work appears to bear out his conclusion that regression of a Brown-Pearce tumor confers immunity on the host, and that a fairly practical method of producing a tumor which, in the great majority of instances, will regress, is furnished by the intradermal route. Certain points are raised, however, which make it difficult to evaluate these results correctly.

Contrary to Besredka's experience of 100 per cent takes by the intratesticular route, nearly a quarter of

the untreated animals used as controls failed to develop intratesticular growths. The breed of rabbit used might have been at fault, or the cause might have lain in part in technical difficulties since of animals refractory to primary inoculation nearly a third of those injected a second time developed takes verified at autopsy. Seasonal variation in the incidence of takes as previously reported (6, 7) was noted. These factors make it difficult to furnish an accurate estimation of the efficacy of any method of immunization. Furthermore, since a considerable number of testicular growths regress spontaneously, claims (14) that a tumor already established may be affected by subsequent treatment can be evaluated only if the experiments are rigidly controlled with a large number of animals.

As previously mentioned, in spite of pathological and immunological studies the nature of the immunity engendered by a regressing or regressed lesion remains obscure. The usual reaction of the host's tissue to injected tumor cells appears to be so altered that material inoculated later is unable to gain the customary foothold. That this in part at least may be a nonspecific reaction is suggested by the evidence that a certain degree of immunity can be obtained by the injection of homologous normal tissues. A considerably higher percentage of immune animals is obtained, however, if the immunizing agent consists of tumor cells, a fact which strongly suggests that specific factors are in part, at least, involved.

The recent demonstration (11) of a complement fixing antibody in the blood of rabbits bearing the Brown-Pearce tumor, even though its presence or absence appears to bear no relation to the ultimate fate of the tumor, offers some encouragement to further investigations along classical immunological lines. Tissue culture methods may permit the study of "tissue resistance" factors, which are ill defined at present.

Summary

1. In confirmation of Besredka's reports, it was observed that over 90 per cent of intradermal Brown-Pearce carcinomas regressed spontaneously, and that rabbits with intracutaneous tumors, or in which these tumors had regressed, were refractory to subsequent implantations of the tumor.

2. Pathological and immunological studies shed no light on the fundamental nature of this immunity. The possibility of its being a nonspecific tissue reaction is discussed.

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The Effect of Prolonged X-Radiation on the Congo Red Index of Rabbits

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In previous experiments (4-6) the effect of prolonged treatment with carcinogenic hydrocarbons on the Congo red index of rabbits was investigated. It was of interest to examine whether other agents capable of producing malignant tumors provoke changes in the Congo red index comparable with those induced by carcinogenic hydrocarbons, and hence the effect of repeated x-radiation on rabbits over a long period was tested. Bloch (2) showed that, in the rabbit, x-rays alone can produce epithelioma, and Lacassagne (8), Lacassagne and Vinzent (9), Burrows, Mayneord and Roberts (3) showed that in combination with a second factor, x-rays can produce sarcoma also.

It is preferable to use purely descriptive terms to express the results of these experiments, and no attempt is made here to discuss the question, to what extent the rate of disappearance of a dye expresses the functional activity of the reticulo-endothelial system. The term *removal factor* is used below to denote the agencies affecting the rate of disappearance of intravenously injected Congo red from the circulation, and this factor may be either positive (more rapid elimination) or negative (slower elimination).

The effect of x-radiation was followed, as in the earlier experiments with carcinogenic hydrocarbons, in 2 ways; namely, by observing (a) the change in the elimination of Congo red injected into the circulation of the rabbit treated with x-rays (primary rabbit) and (b) the effect of injection of extracts of blood from the primary rabbit on the Congo red index of another rabbit (secondary rabbit).

Methods

The Congo red index (C.R.I.) was determined in young rabbits of either sex, weighing from 2 to 2.5 kg., by the Stern-Willheim (11) modification of the method of Adler and Reimann (1); the details of the technic followed are given in the previous paper (6). The results are so calculated that the higher the figure representing the index the more rapid is the removal of the dye. The spontaneous changes of the Congo red index of rabbits during a long period do not exceed ±9 per cent, hence preparations which do not provoke any change greater than this are called indifferent. Responses beyond this level were taken as positive and negative effects respectively. The index was determined in the primary rabbit twice before the irradiation began and during the first year the examination was repeated, first at intervals of 2 to 3 weeks, and later on of 6 to 8 weeks.

Table I: Comparison of Effect upon Secondary Rabbit of Plasma, and of Ultrafiltrate, from Blood of Radiated Rabbit

Experiment	Preparation of blood	Congo red index: Before injection	Congo red index: 24 hrs. after injection	Per cent of change
1.	Plasma	43.5	50.5	+ 16
	Ultrafiltrate	39	52.5	+ 34.6
2.	Plasma	41	49	+ 19.5
	Ultrafiltrate	42	55	+ 30.9
3.	Plasma	40.6	50	+ 23
	Ultrafiltrate	39.8	51	+ 28.1
4.	Plasma	40	40	...
	Ultrafiltrate	43	30	— 30.2
5.	Plasma	47	42	— 10.6
	Ultrafiltrate	39	33.5	— 14.1
6.	Plasma	43	35	— 18.5
	Ultrafiltrate	42.5	37	— 12.9
7.	Plasma	44	36	— 18.3
	Ultrafiltrate	44.5	40	— 10.1
8.	Plasma	40.5	40	— 1.2
	Ultrafiltrate	43.5	40	— 8.1
9.	Plasma	43	30	— 30.3
	Ultrafiltrate	40.5	34	— 16
10.	Plasma	42	38	— 9.5
	Ultrafiltrate	44.5	40.5	— 9

To examine the effect of blood extracts of the primary rabbits upon the secondary rabbits, blood was drawn from the ear vein of the primary rabbits in most cases once before irradiation began and in all cases at intervals of not more than 6 to 8 weeks. The method of treatment of blood used was changed in the course of the experiment; at first protein-free ultrafiltrates from blood plasma, as described previously (6), were used, later on the citrated plasma of the primary rabbit was injected (Table I). Injections of

the whole plasma of primary rabbits caused, as do protein-free ultrafiltrates, an increase or decrease of the elimination of Congo red from the circulation of the secondary rabbit (positive or negative removal factor). In the case of extracts containing positive removal factor the ultrafiltrate seems somewhat more active than whole plasma, while no constant difference could be observed with blood containing negative removal factor.

irradiation on the C.R.I. of the primary rabbits. In some animals the reaction to the first irradiation is an increase in the rate of elimination of Congo red, in others however a decrease in the C.R.I. occurred. This decrease after the first exposure is shown by all the animals which died except 1 (H) in which there was an insignificant rise, but 2 animals with an initial decrease survived. All the rabbits show fluctuation in the C.R.I. throughout the year, amounting to between

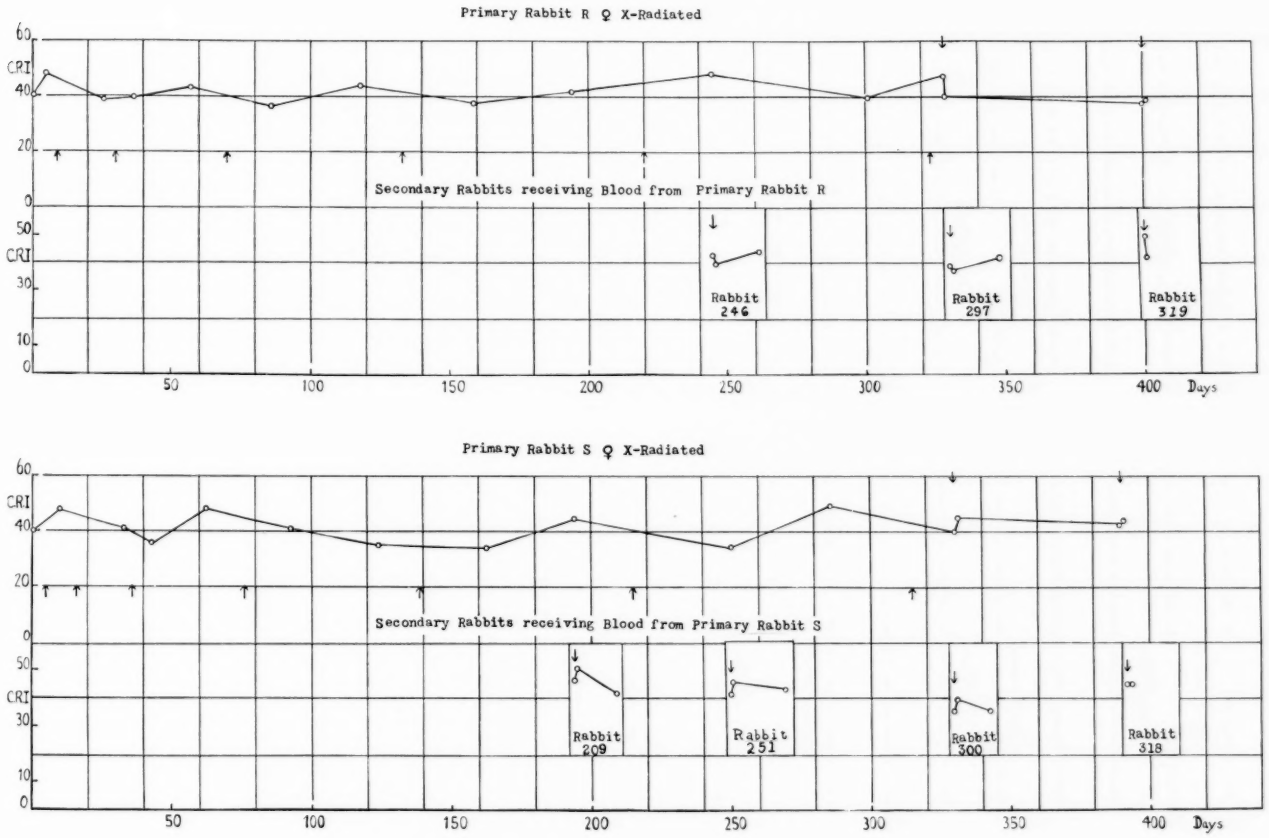

FIG. 1.—Changes of the Congo red index and of the circulating removal factor in the blood of rabbits treated with x-radiation. The arrows pointing upward indicate x-radiation (150 r) of the primary rabbits; those pointing downward indicate the injection of blood extracts into the primary or secondary rabbits.

The whole body of the rabbit was irradiated, the head being covered by lead (tube distance 55 cm., 0.5 mm. copper filter, 140 kv., 5 ma.); the dose was applied in equal parts to both sides of the body. Rabbits receiving the higher doses (500 r) died 2 to 5 days after irradiation. The mortality among those receiving only 150 r in one irradiation was high also; 4 out of the 10 rabbits died during the first month but the surviving animals remained in good general health. No death occurred after the first month. At first the interval between irradiations was 1 to 4 weeks according to the general health of the rabbit. Later on the interval was prolonged, so that the total dosage was between 900 and 1050 r in the course of a year.

Figures 1 to 3 and Table II show the effect of

10.5 and 20 units around the base-line. It was found previously (6), that in about the same length of time the fluctuations of the C.R.I. in untreated rabbits or in rabbits treated with noncarcinogenic hydrocarbons are between 5 and 7 units while in animals treated with carcinogenic hydrocarbons these variations are between 19 and 28 units. The Congo red indices of the x-rayed rabbits show more fluctuation than do those of rabbits untreated or treated with noncarcinogenic hydrocarbons, but less than those of animals treated with carcinogenic hydrocarbons.

Blood extracts; *i.e.*, plasma or ultrafiltrate, of healthy untreated rabbits injected into another rabbit provoke an increase in the C.R.I. in this animal in 24 hours (positive removal factor). If the primary rabbit be

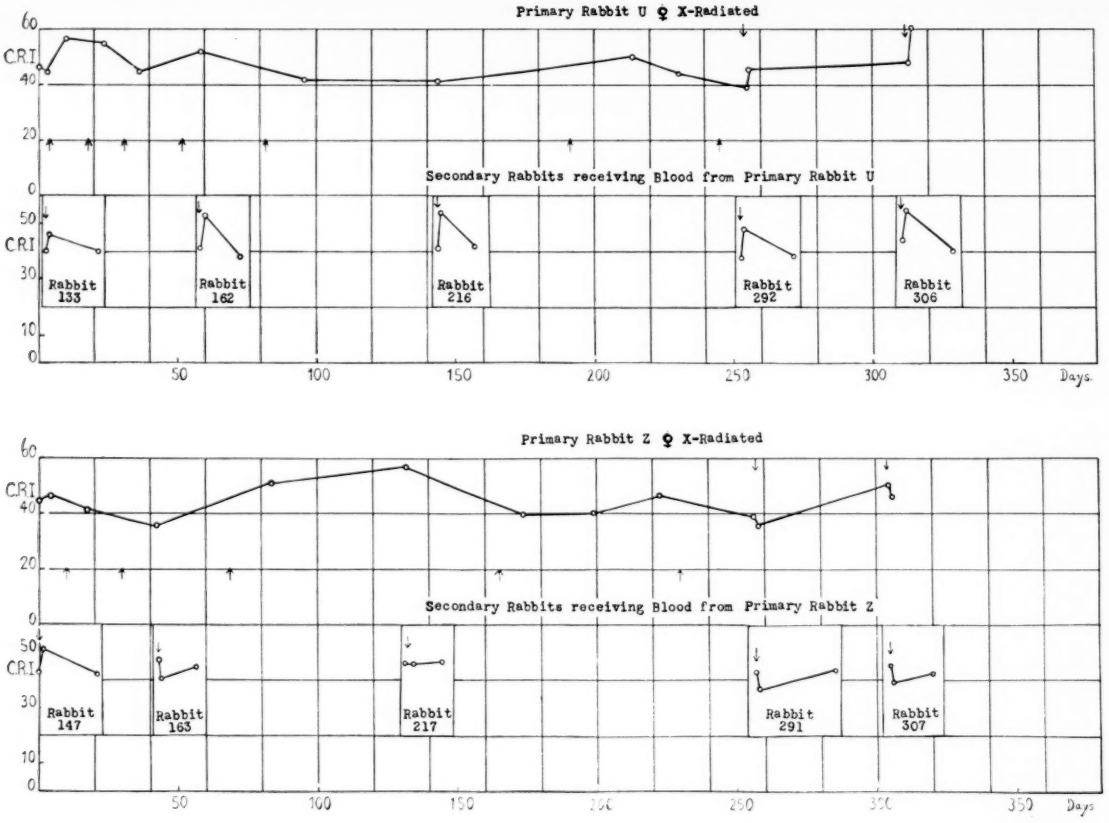

FIG. 2.—Changes of the Congo red index and of the circulating removal factor in the blood of rabbits treated with x-radiation. The arrows pointing upward indicate x-radiation (150 r) of the primary rabbits; those pointing downward indicate the injection of blood extracts into the primary or secondary rabbits.

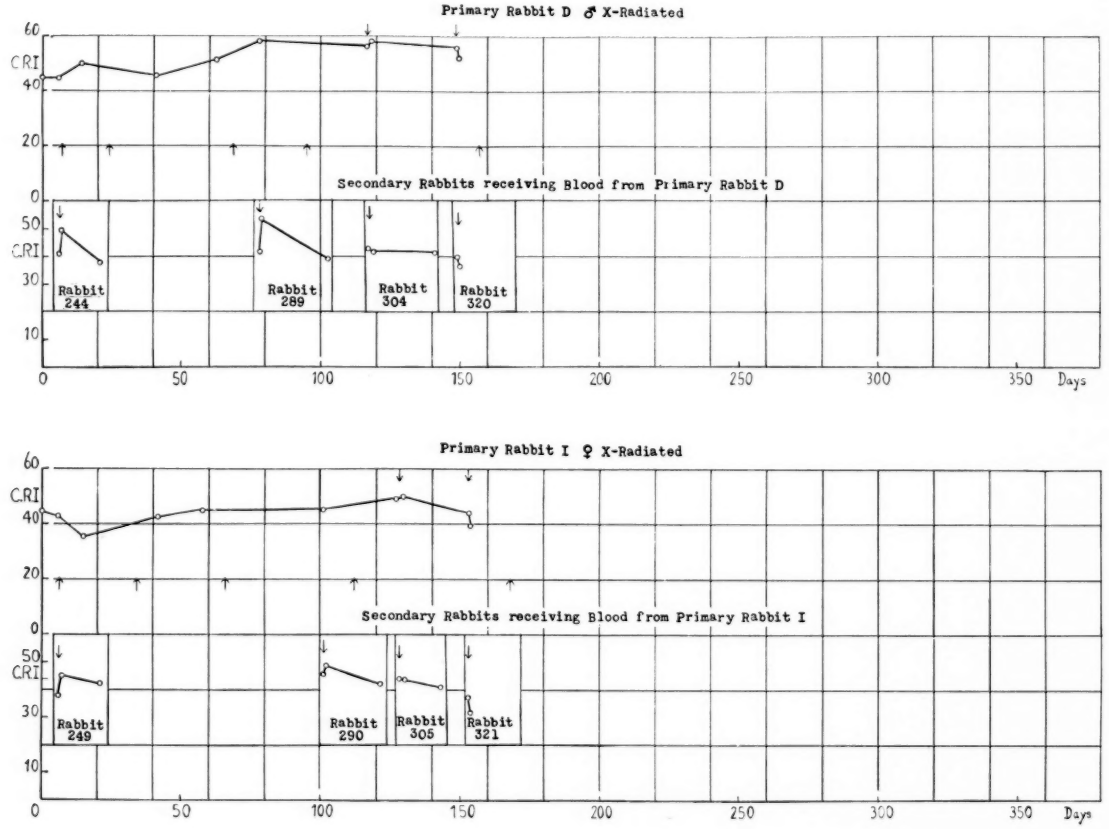

FIG. 3.—Changes of the Congo red index and of the circulating removal factor in the blood of rabbits treated with x-radiation. The arrows pointing upward indicate x-radiation (150 r) of the primary rabbits; those pointing downward indicate the injection of blood extracts into the primary or secondary rabbits.

treated with carcinogenic hydrocarbons this effect of blood extract is changed in such manner that the majority of samples of blood have either no significant effect, or cause a decrease in the rate of elimination of Congo red (negative removal factor). The blood of x-ray-treated rabbits (Table III) furnished extracts almost wholly indifferent or negative in effect in 5 out of 6 animals; in 1 rabbit (U), however, the removal factor remained positive after the administration of 900 r in the course of a year.

The presence of the removal factor in the blood of the primary rabbits was tested in the later course of the experiment not only in a secondary rabbit, but also by re-injection of the plasma into the same rabbit. With one exception the primary rabbit reacted to its own plasma in the same way as did the secondary rabbit, both rabbits showing positive or negative responses simultaneously (Table IV). The reaction of the primary rabbit to the re-injection of its own blood extract is independent of the actual state of the C.R.I.; rabbits with high C.R.I. may react with an increase or decrease in the rate of elimination of the dye. It seems that

TABLE II: EFFECT OF X-RADIATION UPON THE CONGO RED INDEX OF PRIMARY RABBITS

Rabbit	Congo red index: Before irradiation		After irradiation started, time in weeks											Range of variation
	1 week	Immediately	1-2	3-4	5-6	7-9	11-13	15-17	21-23	28-30	33-35	41-43	44-47	
R	..	40.5	50	39	39.5	44	37.5	44	38.5	44	48	40	47.5	12.5
S	..	40	47.5	41.5	35.5	48	41	35.5	35	45	34.5	49	40.5	14.5
T	47	45	37	42.5	..	Died	..	..	..	..	..	..	..	..
U	46.5	44.5	57	55	45	52	42	..	41	50	44	39	44	18
V	41	44.5	34.5	31	40	Died	..	..	..	..	..	..	..	..
Z	44.5	46.5	41.5	36	..	..	51	56	40	40.5	47	39.5	50	20
C	41	44	35	Died	.	..	..	..	..	..	..	..	..	..
D	45	44.5	50	45	..	51	58	56	55.5	..	..	..	..	10.5
H	35	35.5	38.5	36.5	Died	..	..	..	..	..	..	..	..	..
J	44.5	42.5	35.5	42.5	45	..	45	49.5	44	..	..	..	..	14

TABLE III: EFFECT OF BLOOD OF PRIMARY RABBITS ON CONGO RED INDEX OF SECONDARY RABBITS

	Primary rabbit U					Primary rabbit Z					Primary rabbit D				
	No. of secondary rabbit	Congo red index I	II	III	IV	No. of secondary rabbit	Congo red index I	II	III	IV	No. of secondary rabbit	Congo red index I	II	III	IV
Before primary rabbit irradiated	133	40.5	46	40	+ 13.6	147	43	51.5	42	+ 19.8	244	41	49.5	38	+ 20.6
After irradiation started:															
4- 6 weeks	162	41.5	54	38	+ 30	163	47.5	40.5	44.5	— 14.6	..	..	..	..	..
11-13 weeks	..	..	..	..	..	..	..	..	..	..	289	41.5	53.5	39	+ 28.9
15-17 weeks	..	..	..	..	..	217	45	45	45.5	0	304	42.5	42	40	+ 1.1
20-24 weeks	216	41.5	54	42	+ 30	..	..	..	..	..	320	39	37	..	— 5.1
34-36 weeks	292	37.5	50	38	+ 33.4	291	43.5	37.5	44	— 13.8	..	..	..	..	..
42-47 weeks	306	44	54.5	40	+ 23.8	307	45.5	39.5	42.5	— 13.2	..	..	..	..	..
52-65 weeks	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..

	Primary rabbit I					Primary rabbit R					Primary rabbit S				
	No. of secondary rabbit	Congo red index I	II	III	IV	No. of secondary rabbit	Congo red index I	II	III	IV	No. of secondary rabbit	Congo red index I	II	III	IV
Before primary rabbit irradiated	249	38	45.5	42	+ 19.6	..	..	..	..	..	..	..	..	..	..
After irradiation started:															
4- 6 weeks	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
11-13 weeks	290	46.5	49	42	+ 5.4	..	..	..	..	..	..	..	..	..	..
15-17 weeks	305	44	44	40.5	0	..	..	..	..	..	..	..	..	..	..
20-24 weeks	321	38.5	32	..	— 16.9	..	..	..	..	..	209	47	51	40	+ 8.5
34-36 weeks	..	..	..	..	..	246	43	40	44.5	— 6.9	251	42	46	..	+ 9.5
42-47 weeks	..	..	..	..	..	297	39.5	39	42	— 1.2	300	36.5	40	36	+ 9.5
52-65 weeks	..	..	..	..	..	319	50	42.5	..	— 15.0	318	45	45	..	0

Column I. C.R.I. before injection of 2 cc. blood extract from primary rabbit.
Column II. C.R.I. 24 hours after injection.
Column III. C.R.I. 14 to 20 days after injection.
Column IV. Difference between I and II, expressed as percentage of I.

the removal factor of the blood does not act as long as it is in the circulation, but develops either through changes occurring on withdrawal, or after subcutaneous administration.

TABLE IV: COMPARISON OF THE EFFECT OF PLASMA UPON PRIMARY AND SECONDARY RABBITS

I = Re-injection of 2 cc. plasma into the primary rabbit.
II = Injection of 2 cc. plasma into a secondary rabbit.

Rabbit		Congo red index: Before injection	Congo red index: 24 hrs. after injection	Per cent of change
S	I	40.5	45	+ 11.1
	II	36.5	40	+ 9.8
S	I	45	45	..
	II	45	45	..
R	I	47.5	44	— 7.4
	II	39.5	39	— 1.3
R	I	38	38.5	+ 1.3
	II	50	42.5	— 15
U	I	39	45.5	+ 16.7
	II	37.5	50	+ 33.4
U	I	48	61.5	+ 28.2
	II	44	54.5	+ 23.8
Z	I	39.5	36.5	— 7.6
	II	43.5	37.5	— 16
Z	I	50	47.5	— 5
	II	45.5	39.5	— 13.2
D	I	56	57.5	— 2.8
	II	42.5	42	— 1.1
D	I	55.5	52	— 6.3
	II	39	37	— 5.1
I	I	49.5	50.5	— 2.1
	II	44	44	..
I	I	44	39.5	— 10.2
	II	38.5	32	— 16.8

DISCUSSION

The investigations of earlier workers were restricted to observation of the immediate effect of x-radiation on the phagocytic capacity of the reticulo-endothelial system, their experiments being carried out during periods from a few hours up to 5 days (7). The technic employed was usually that of vital staining and only Schürer (10) used the method of Adler and Reimann. He found a decrease followed by an increase in the rate of elimination of Congo red during the first 48 hours. When the rate of disappearance of a dye from the circulation is under observation one would be able to demonstrate either positive, negative, or no effects by varying the time of single observations. The results recorded here show that, in the course of 1 year, x-radiation of the rabbit induces changes in the rate of disappearance of Congo red from the circulation and likewise in the circulating removal factor. These changes are similar to those produced by injection of carcinogenic hydrocarbons, but differ in degree from them. The fluctuations of the C.R.I. observed during 1 year do not reach the range of those produced by carcinogenic hydrocarbons. All rabbits treated with carcinogenic compounds showed the appearance of a negative removal factor in their blood in several samples (6). Although the positive removal factor disappeared from the circulation of x-rayed rabbits in 5 cases, in only 1 animal (Z) could the negative removal factor be demonstrated repeatedly. Moreover 1 rabbit (U) failed to show any changes in the removal factor. If one considers the changes in the Congo red index and in the removal factor in rabbits treated with carcinogenic hydrocarbons as a change connected with the carcinogenic property of these compounds, one would be inclined to explain the changes provoked by irradiation on the same lines, and to class x-rays among the slightly carcinogenic agents.

SUMMARY

Treatment with x-rays continued for 1 year induced fluctuations in the Congo red index of the rabbit which range between those of normal rabbits and those seen in rabbits treated with carcinogenic hydrocarbons.

Five out of 6 rabbits showed changes in the circulating removal factor resembling those seen in animals treated with carcinogenic hydrocarbons, while in 1 rabbit the removal factor retained the normal positive character.

I am much indebted to Dr. J. Iball and to Dr. E. M. F. Roe for carrying out the treatment with x-radiation.

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Stimulation of Tumor Induction by an Inhibitor of Cell Glycolysis

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In another communication (3) evidence was given that substances which reversibly inhibit the glycolytic process of cells also inhibit the rate of tumor induction by chemical carcinogens. By using a series of non-hydrolyzing chlor-compounds soluble in fat and water, it was shown that their power of inhibiting the rate of glycolysis ran parallel to their power of inhibiting the rate of tumor induction in mice by 3,4-benzpyrene and 1,2,5,6-dibenzanthracene. The most potent of the substances used was mono-chloracetone, and this substance has been used exclusively in the experiments described in this paper.

In the experiments already reported this chlor-compound had been applied in a concentration and at time intervals arbitrarily chosen; *viz.*, 0.3 per cent thrice weekly. The question arose whether the degree of inhibition of the rate of tumor induction was a function of these factors and might be modified by their variation. It is certainly true that higher concentrations increase the rate of glycolytic checking *in vitro,* and, if pushed too far, inflict irreversible damage on cells, but such simple relations could not be assumed for the inhibitory effect on tumor induction *in vivo.* In fact, Experiments I and II show that higher concentrations and more frequent applications of the chlor-compound tend to annul the inhibitory effect, and even suggest that some slight degree of stimulation can be effected.

More clear-cut evidence for a stimulatory effect is given in Experiments III and IV, in which the chlor-compound was applied after discontinuing a preliminary treatment with the carcinogen.

Considering the experiments as a whole, it has been demonstrated that the same substance, a simple inhibitor of cell glycolysis, can, under different experimental conditions, either retard or stimulate the rate of in-induction of tumors.

Experiment I.—Effect of increased amounts of mono-chloracetone on the rate of tumor induction.—One hundred and twenty mice of mixed stock were epilated in the interscapular region 2 days before the experiment was begun, but not subsequently. Sixty of these mice were treated twice weekly with 0.3 per cent 3,4-benzpyrene and 60 similarly with 0.1 per cent benzpyrene, the solvent used being 98 per cent ether, which had been kept in the dark over sodium wire, plus 2 per cent medicinal liquid paraffin. The local and systemic damage caused by benzene is avoided by the use of this solvent; moreover it has been shown (2) to be more effective than benzene, as evidenced by the shorter average induction time for tumors in mice.

Thirty mice from each group of 60 were treated, over the same period, with 0.5 per cent chloracetone in the same solvent 6 times weekly. This represents, in the group in which 0.3 per cent benzpyrene was used, a weekly application more than 3 times greater than that employed in the earlier experiments, and, in the case where 0.1 per cent benzpyrene was used, an increase in the ratio chloracetone/benzpyrene to 10 times. The remaining 30 mice from each group acted as controls, receiving benzpyrene alone.

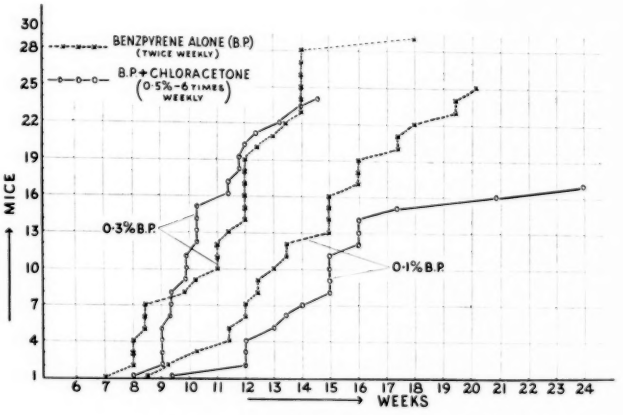

FIG. 1.—Showing effect of 0.5 per cent mono-chloracetone on the rate of tumor induction by 0.3 and 0.1 per cent solutions of 3,4-benzpyrene.

The experimental results are shown in Fig. 1. Each symbol on this graph represents the time when a papilloma was first noticed in an individual mouse; the curve formed by joining these points for a particular group shows, at a glance, the time-course of tumor induction for that group.

It is clear that the inhibitory effect of chloracetone found in the earlier experiments has here been abolished by the use of the higher concentration and

more frequent applications. In 3 of the groups the percentage of surviving mice was high, but in the group treated with 0.1 per cent benzpyrene plus chloracetone more than 40 per cent of the animals died during the course of the experiment. The apparent slight inhibition in this group is probably unreal, being determined by the high mortality. The experiment demonstrates, however, in a qualitative manner that larger amounts of chloracetone do not produce larger inhibitions, but tend to influence the process of tumor development in the opposite direction. Had this been an isolated experiment, the obvious conclusion would have been that chloracetone was innocuous.

The average induction times of papilloma formation in the 4 groups are given in Table I.

TABLE I: AVERAGE INDUCTION TIMES IN MICE TREATED WITH BENZPYRENE AND BENZPYRENE PLUS CHLORACETONE

Treatment: Benzpyrene twice weekly; Chloracetone 6 times weekly	No. of mice bearing tumors; *i.e.*, all survivors	Average induction time of papillomas
0.3% Benzpyrene alone	29	11 wks. 3 days
0.3% Benzpyrene plus Chloracetone (0.5%)	24	10 wks. 5 days
0.1% Benzpyrene alone	25	14 wks. 3 days
0.1% Benzpyrene plus Chloracetone (0.5%)	17	15 wks. 1 day

TABLE II: EFFECTS OF DIFFERENT CONCENTRATIONS OF CHLORACETONE ON TUMOR INDUCTION TIMES

Treatment: Benzpyrene twice weekly; Chloracetone 3 times weekly	No. of mice bearing tumors; *i.e.*, all survivors	Average induction time of papillomas
0.3% Benzpyrene alone	28	11 wks. 4 days
0.3% Benzpyrene plus 0.3% chloracetone	24	17 wks. 0 days
0.3% Benzpyrene plus 3% chloracetone	24	12 wks. 5 days

When 0.3 per cent benzpyrene was used, the average induction time for the chloracetone-treated mice was slightly lower than that of the controls, suggesting a mild stimulatory action, though the difference is probably not statistically significant.

Experiment II.—Direct comparison of the differential effect of large and small concentrations of chloracetone on the rate of tumor induction.—In order to make certain that the above experimental results were not fortuitous, the effects of large and small amounts of chloracetone were studied in a single experiment. Ninety mice were treated twice weekly (Tuesdays and Thursdays) with 0.3 per cent benzpyrene as before. Thirty were treated also, thrice weekly (Mondays, Wednesdays, and Fridays), with 0.3 per cent chloracetone; 30 were treated in the same way with 3 per cent chloracetone; and 30 were kept as controls. Fig. 2 shows the results of this experiment.

From these results two points may be noted: (a) The lower concentration, 0.3 per cent, of chloracetone strongly inhibited the rate of papilloma formation, thus confirming the earlier work. (b) The higher concentration, 3 per cent, of chloracetone certainly caused no general inhibition, but seemed to stimulate papilloma formation slightly in the more susceptible mice, and to retard it slightly in the less susceptible. The net effect on the average induction time was negligible, as is shown in Table II.

Experiment III.—Chloracetone as a stimulator of tumor induction by benzpyrene.—The question arose whether all the preliminary treatment with chloracetone during the full latent period was necessary to demonstrate the known inhibiting powers of this substance on the process of tumor induction. If the early phases of cell response to carcinogens are conceived as a succession of reversible reactions, discontinuous in nature, then an inhibiting agent might well exercise its power if its application were commenced only towards the end of the latent period. Or, alternatively, if applied subsequent to a limited treatment with carcinogen, just below that necessary to induce papilloma formation, it might entirely prevent the emergence of tumor cells. The latter would be equiva-

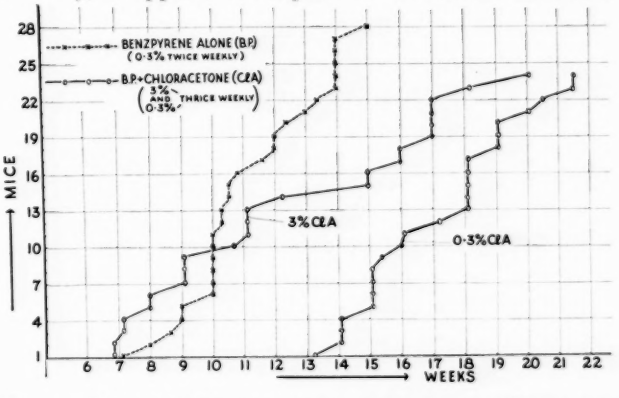

FIG. 2.—Effect of different concentrations of mono-chloracetone on the rate of tumor induction by 3,4-benzpyrene.

lent to a prophylactic treatment of a precancerous condition of the skin. It is known from the work of Leitch (6) that when the application of a carcinogen is discontinued before external signs of neoplastic reaction are visible to the naked eye, a certain percentage of animals will develop either temporary or permanent papillomas, or even occasional malignant tumors.

To investigate this second possibility, 80 mice, after one epilation, were treated in the usual way with 0.3 per cent benzpyrene for 8 weeks, when the earliest papillomas could be expected to appear. The application of carcinogen was then discontinued. Forty mice

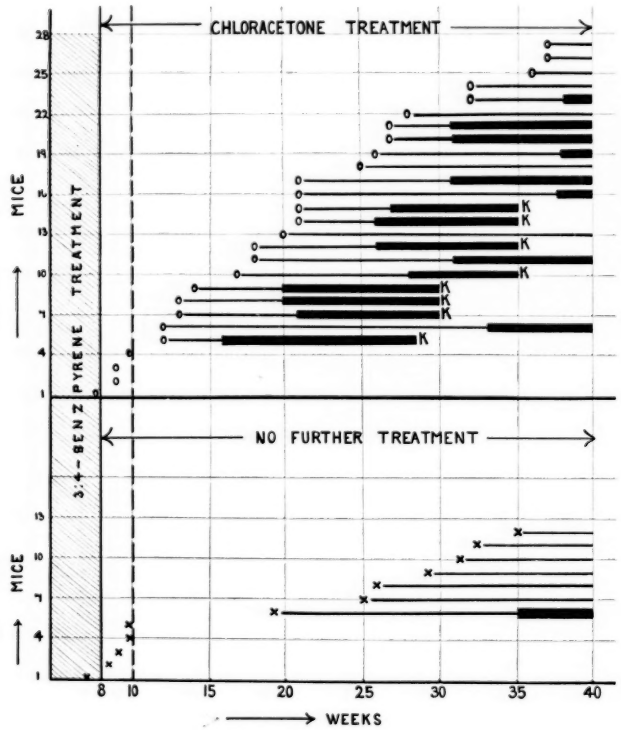

FIG. 3.—Stimulation of tumor induction by 0.3 per cent monochloracetone after preliminary treatment with 3,4-benzpyrene.

received no further treatment and were examined at weekly intervals for tumor development. The remaining 40 were treated thrice weekly with 0.5 per cent chloracetone.

The result of this experiment is shown in Fig. 3.

In both the control and the chloracetone-treated groups, 1 papilloma appeared before the benzpyrene applications were discontinued, and 3 or 4 more emerged during the next 2 weeks. It is assumed that these latter were incipient or not clearly visible at the end of 8 weeks. The broken line at 10 weeks in Fig. 3 indicates the end of this early phase of papilloma formation. The omission of these papillomas in assessing the subsequent course of the experiment is further justified by the fact that the next papilloma in the control group appeared only at the 19th week.

Eliminating these, and considering only the survivors after 40 weeks, including those which had been killed when bearing epitheliomas, the experimental results are summarized in Table III.

Though this experiment was conceived as a possible means of restoring a precancerous condition to normality by an agent proved to possess inhibiting powers on the process of carcinogenesis, precisely the opposite result was obtained. The manifest stimulatory action was most impressive at the 29th week, when, as against 3 small papillomas in the control group, there were 18 growing tumors, including 8 epitheliomas, in the chloracetone-treated group. Moreover, most of the papillomas in the control group showed little sign of growth up to 40 weeks, in contrast to the rapid growth of those in the chloracetone-treated group.

Experiment IV.—Attempt to increase the stimulating action of chloracetone by larger doses.—This experiment was essentially a repetition of Experiment III, except that 3 per cent chloracetone was applied thrice

TABLE III: EFFECT OF CHLORACETONE APPLIED AFTER CESSATION OF PAINTING WITH BENZPYRENE

Mouse group	No. of survivors after 40 weeks	Total no. of tumors	Papillomas	Epitheliomas
Control	29	7	6	1
Chloracetone-treated	30	23	7	16

weekly, thus making the weekly dose 3 times greater than that used in Experiment III. It was thought possible that some difference of reaction might be found by analogy with the differential effect of concentration shown in Experiments I and II. But this was not so. The experiment had to be concluded after 28 weeks, but up to that time increased amounts of chloracetone effected a stimulation not detectably different from that in Experiment III. The results are summarized in Fig. 4.

DISCUSSION

The present experiments, taken together with those described previously (3), demonstrate that the rate of tumor induction can be retarded or accelerated by a substance which has the characteristic biochemical property of inhibiting cell glycolysis. The final biological result depends upon the concentration of the compound used and the time of its application in relation to that of the carcinogen. When these times overlap, low concentrations produce large inhibitory effects, while higher concentrations annul this power of inhibition, thus appearing to behave innocuously. When these times are consecutive; *i.e.,* when the inhibitor of glycolysis is applied after discontinuing the preliminary treatment with the carcinogen, the compound produces strong stimulatory effects which are independent of the concentration used.

The conclusion from the earlier experiments that the power of inhibiting cell glycolysis and the degree of inhibition of the rate of tumor induction run parallel, is clearly only one facet of the truth. The present experiments make it necessary to abandon the notion of such simple relationships.

The original conception that it might be possible to retard or accelerate tumor induction by known, but presumably different, types of metabolic disturbance could hardly be used in explaining the fact that a single substance, under somewhat different experimental conditions, is able to influence the same biological process in opposite directions.

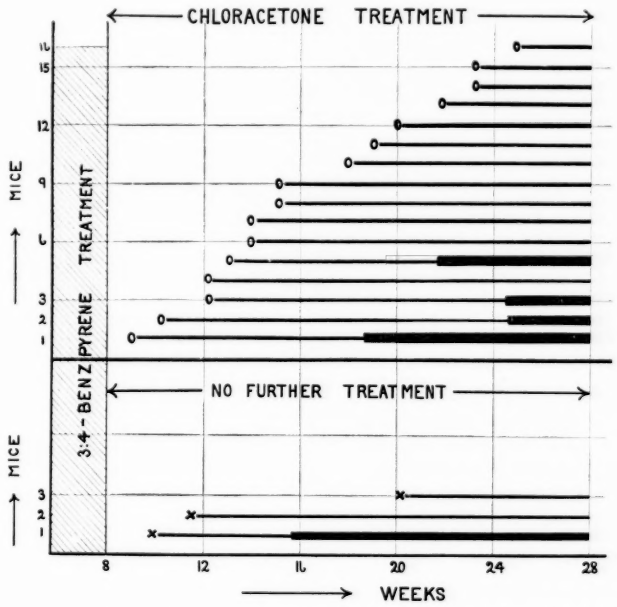

FIG. 4.—Stimulation of tumor induction by 3 per cent monochloracetone after preliminary treatment with 3,4-benzpyrene.

In Experiments III and IV, it will be noticed that the spread in time of tumor induction in the chloracetone-treated series is greater than would be the case if 0.3 per cent benzpyrene had been applied continuously. Such a result might be anticipated if a preliminary treatment with a potent carcinogen had been followed by treatment with a milder carcinogenic agent. Indeed, Hieger (4) has studied the summation effect of 2 carcinogens in an experiment very similar to these. He applied benzpyrene in the same concentration, at the same intervals, and for the same period, as in Experiments III and IV, and followed this by treatment with 1,2,5,6-dibenzanthracene. Though the number of induced tumors was small, he concluded that an additive effect of the two carcinogens had been demonstrated. An analogous experiment by Berenblum (1), in which the 2 carcinogenic agents, tar and solid carbon dioxide, were applied successively, resulted in a summation of their separate effects. This result was discussed in terms of variable intensities of irritation, since when the 2 agents were applied concurrently tumor induction was inhibited. This rather loose conception of subminimum and supermaximum degrees of irritation defies simple analysis, but it would be arresting if benzpyrene and dibenzanthracene applied together over the same period proved to be likewise mutually inhibitory.

By substituting for dibenzanthracene or solid carbon dioxide a simple inhibitor of glycolysis, mono-chloracetone, a much more conclusive result was obtained. But the concept of summation, in an additive sense, is no longer tenable, except by attributing carcinogenic properties to mono-chloracetone, which experiment has shown to be unjustified. The concept of stimulation must here be introduced, in which a residual tendency to carcinogenesis is enhanced by the cooperation of a disturbance of cell metabolism. The nature of this metabolic disturbance may be complex, and the analogy between this checking of glycolysis *in vitro* and the action *in vivo* may not be sufficiently comprehensive.

Experiments III and IV also bear a formal analogy to those of Rous and Kidd (5, 7), with tar and papilloma virus, in which a precancerous condition rapidly becomes cancerous through the intervention of a virus.

No precise conclusions of the relation between metabolic disturbances and tumor induction by chemical carcinogens can be drawn until further experimental data have accumulated.

Summary and Conclusions

It has been shown that the effect of mono-chloracetone, an inhibitor of cell glycolysis, on the rate of tumor induction by 3,4-benzpyrene is a function of the concentration used. Low concentrations cause marked inhibition, but higher concentrations appear to be innocuous, since the net effect on the average induction time is negligible. Higher concentrations may even prove to be slightly stimulatory in mice more susceptible to tumor development.

The same inhibitor of cell-glycolysis is capable, in a wide range of concentrations, of stimulating tumor induction in mice which have received a preliminary, but subeffective, treatment with a chemical carcinogen.

These mixed effects of a substance with a single characteristic biochemical property make clear inferences of the relation between disturbed metabolism and tumor induction impossible until additional data have been accumulated.

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Retardation of the Rate of Tumor Induction by Hydrolyzing Chlor-Compounds

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The effect on the rate of tumor induction of a group of chlor-compounds which reversibly inhibit the glycolytic process of cells has already been described (5, 6). The compounds used were soluble in water and fats, and were nonhydrolyzable under physiological conditions of pH and temperature. The chemical reactivity of their chlorine atoms was the factor which determined the potency of their biological action. But their influence on tumor induction was not simple. Inhibitory or stimulatory effects could be obtained by a single chlor-compound, the final result being determined by the concentration used and also by the time of its application relative to the treatment with carcinogen.

The most active nonhydrolyzing chlor-compound used was mono-chloracetone. It would have been possible to study the effects of still more active halogen compounds of this type; *e.g.*, the bromine and iodine analogues of mono-chloracetone, had not the irritating properties of such compounds precluded their use.

Chlor-compounds, considered from the point of view of increasing chemical reactivity, can be ranged from the nonhydrolyzing types used in the earlier work to types which are so reactive that contact with aqueous media causes hydrolysis. The acid chlorides are familiar examples of this type, and they can be graded according to their rate of hydrolysis. Berenblum (1-3) has focused attention on mustard gas and related compounds which come under the heading of hydrolyzing halogen compounds. In the work reported here, a series of aliphatic, and one aromatic, acid chlorides has been used.

The experiments demonstrate that all these acid chlorides exert an inhibiting influence on the process of tumor induction in the skin of mice by 3,4-benzpyrene. The degree of inhibition by the different members of this class of compounds varies with their physical and chemical characteristics, molecular size and rate of hydrolysis being the variable factors which probably determine their relative effectiveness.

Experiments

1. Using 0.3 per cent 3,4-benzpyrene.—Seven batches of mice, each composed of 15 mixed laboratory stock animals, were epilated in the interscapular region 3 days before the experiment was commenced, but not subsequently. One batch of 15 mice was treated with 0.3 per cent benzpyrene in 98 per cent ether plus 2 per cent liquid paraffin, twice weekly (Mondays and Thursdays). The remaining 6 batches received similar treatment with benzpyrene, and, in addition, were painted with a 1 per cent acid chloride solution, in the same solvent, twice weekly (Tuesdays and Fridays). The hydrocarbon was confined to an area of about 1 sq. cm.; the acid chloride was applied to a considerably larger area to ensure wide overlapping. These applications were continuous over 40 weeks in mice remaining free of tumors. This concentration of acid chloride was chosen quite arbitrarily. Subsequent experiments have shown that considerably lower concentrations act in the same way, though no analysis of the relation between degree of inhibition and concentration applied has been undertaken. Only the qualitative aspect of the experimental findings is emphasized here.

Although most of the mice showed no obvious skin lesions in the painted area, a few suffered from minor wounds as a result of scratching provoked by insect bites. This is unavoidable when applying skin irritants, but in a series of mice treated with the acid chlorides alone, few visible lesions developed, and these healed promptly when the treatment was discontinued. Curves showing the times when papillomas appeared in the various series are reproduced in Fig. 1. The times when malignancy supervened, though noted, cannot be shown by this method of expressing results. They are, in any case, irrelevant for this type of experiment, since the inhibitory action is confined to the latent period. Papillomas, once formed, always grew progressively.

The curves showing the effect of stearyl, acetyl, valeryl, and benzene sulfochlorides only are included in Fig. 1. Those for palmityl and myristyl chlorides are omitted, since they corresponded closely to those for stearyl and valeryl chlorides respectively. Clear inhibitions of the rate of papilloma formation are obvious in all cases, the most pronounced being with benzene sulfonyl chloride. Several factors probably

determine these differential potencies, including molecular size which reflects the power of diffusion and hence the depth of penetration, and the rate of hydrolysis. No exact correlation with a single physical or chemical characteristic seems possible; *e.g.,* the curves for acetyl and benzene sulfochlorides occupy anomalous positions when molecular size is considered, and the curve for acetyl chloride, the most easily hydrolyzed, is again out of step when rates of hydrolysis are considered. Benzene sulfochloride is hydrolyzed much more slowly than all the other acid chlorides, and is a comparatively small molecule. Its enhanced power of inhibiting tumor induction suggests that further low-molecular acid chlorides, having still slower rates of hydrolysis, may be found to exhibit even greater potency.

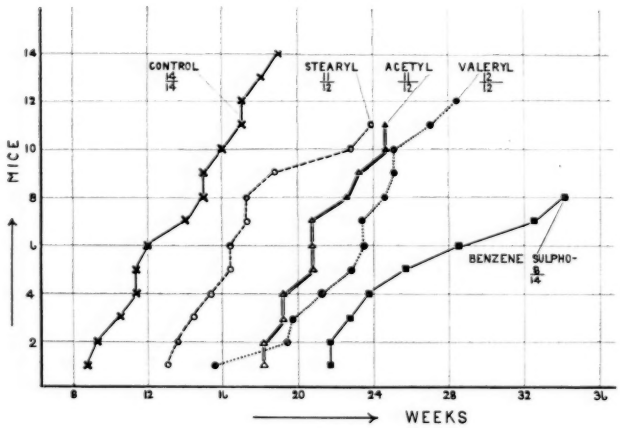

FIG. 1.—Retarding effect of acid chlorides on the rate of tumor induction by 0.3 per cent 3,4-benzpyrene.

II. Using 0.1 per cent 3,4-benzpyrene.—It was thought inadvisable to increase the concentration of the acid chlorides in an attempt to enhance their inhibitory effect. But the converse experiment was carried out. The ratio carcinogen/inhibitor was reduced two-thirds by retaining the same concentration of acid chloride as in Experiment I, and using 0.1 per cent 3,4-benzpyrene. In all other respects the preceding experimental procedure was followed. Details of the result of this experiment are shown in Fig. 2. The curves for palmityl and myristyl chlorides are again omitted from the chart for reasons already given. The curves showing the times of papilloma appearance are here noticeably more spread out along the time axis. The experiment was terminated after 40 weeks when all groups showed almost 100 per cent tumor formation except that treated with benzene sulfochloride.

In this latter group no wart occurred until the 22nd week, at a time when every mouse in the control group bore a tumor. Even after 40 weeks, 43 per cent of the mice treated with benzene sulfochloride were free of papillomas.

Since the mortality rate was very low, and, with one exception, 90-100 per cent of the mice had developed tumors before 40 weeks, significant average induction times for papilloma development can be given (Table 1).

III. Mode of action of hydrolyzing chlor-compounds.—Under test tube conditions in the presence of compounds containing such groups as OH, NH_2, SH, and in strongly alkaline media, the acid chlorides rapidly combine with these active groups. Under physiological conditions of temperature and pH, in Ringer's solution containing, for example, cysteine, manometric experiments have shown that fixation of the thiol group is negligible. Owing to the insolubility of the acid chlorides in aqueous media, a simple hydrolysis predominates.

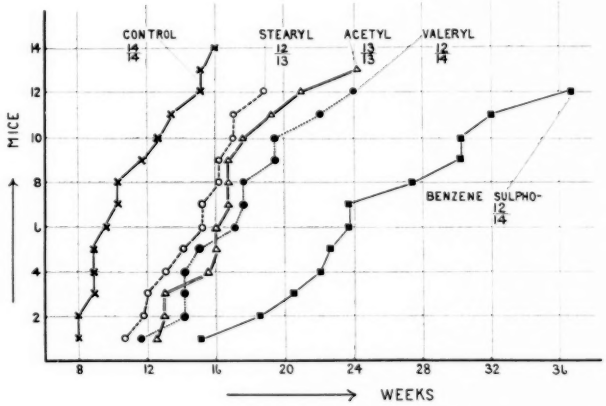

FIG. 2.—Retarding effect of acid chlorides on the rate of tumor induction by 0.1 per cent 3,4-benzpyrene.

TABLE I: AVERAGE INDUCTION TIME FOR PAPILLOMAS IN MICE TREATED WITH BENZPYRENE AND ACID CHLORIDES

Treatment	Average induction time: 0.3% benzpyrene	Average induction time: 0.1% benzpyrene
Benzpyrene alone	11 weeks 2 days	14 weeks 1 day
Benzpyrene + stearyl chloride	14 weeks 5 days	17 weeks 2 days
Benzpyrene + palmityl chloride	14 weeks 5 days	16 weeks 3 days
Benzpyrene + myristyl chloride	16 weeks 0 days	22 weeks 6 days
Benzpyrene + valeryl chloride	17 weeks 3 days	22 weeks 2 days
Benzpyrene + acetyl chloride	16 weeks 6 days	21 weeks 3 days
Benzpyrene + benzene sulfochloride *	25 weeks 2 days	26 weeks 4 days

* 43 per cent of surviving mice had not developed papillomas after 40 weeks treatment.

When acid chlorides are applied to the skin of mice, there is probably a twofold effect: first, a simple hydrolysis with liberation of HCl and the corresponding acid; second, a fixation, by chemical combination, of groups containing labile hydrogen atoms. The relative

extent of these 2 possible reactions is obviously governed by a variety of factors, the chief of which is the rate of hydrolysis of the chloride used. Exact data to decide this point are not available, but evidence from experiments *in vitro,* given later when considering the effect of the acid chlorides upon glycolysis, points strongly to the probability that simple hydrolysis is the predominant reaction in the presence of living cells.

This liberation of HCl within the cells implies profound local changes of pH, with concomitant disturbances of all cell equilibria. Such damage, if slight, is followed by recovery when adequate intervals are allowed between successive applications. The old conception that superoptimal "irritation" proves antagonistic towards the process of carcinogenesis has often been used to explain this retarding effect of nonspecific irritants. Discussion on these lines is sterile unless the word "irritation" is more precisely defined. In the experiments previously described, the "irritants" used were specific inhibitors of glycolysis. Can the hydrolyzing chlor-compounds be regarded as falling into the same category?

IV. Effect of acid chlorides on the glycolytic process.—The normal technic for studying the effect of substances on metabolic processes *in vitro* consists in adding the substance to the saline medium in which a thin slice of tissue is suspended. A fall of glycolytic rate, for example, is observed over a suitable interval, and on returning the tissue slice to normal physiological media, the initial rate of glycolysis returns; *i.e.,* the action is reversible. All the chlor-compounds used in earlier work exerted this type of effect, and when the test was made with tumor tissue, the viability of the cells was unimpaired after 3 hours of treatment at suitable concentrations, as proved by 100 per cent positive transplantations.

When the acid chlorides are tested in this way little or no inhibition of glycolysis is observed, since the compounds are rapidly hydrolyzed in the saline medium, the net result being a lowering of pH to an extent determined by the amount of chloride used. Since the curve showing the relation between rate of glycolysis and the pH of the medium is well known from the work of Warburg, Posener, and Negelein (7), it is possible to predict with fair accuracy the fall in glycolytic rate which will accompany the addition of known amounts of these hydrolyzing chlor-compounds to the medium. In fact, the addition of an equivalent amount of HCl is equally effective in producing the same degree of inhibition. This result is conditioned by the fact that little or none of the acid chloride used comes into direct contact with the living tissue.

To overcome this difficulty it is possible to add the chlorides directly to minced tissue, with thorough mixing. This method was employed by Berenblum *et al.* (4) when studying the effect of mustard gas on cell metabolism. The method has many drawbacks when highly active compounds are used, because of uneven distribution of the added agent. The net change observed in the rate of any metabolic process can represent only a rough average, and high local concentrations can introduce destructive effects which make the concept of specific action meaningless. The kind of result obtained is illustrated by the following experiments:

Five grams of Jensen rat sarcoma tissue were finely minced and 0.08 cc. of myristyl chloride was added and well mixed by further mincing with scissors. A suitable amount of this mince was weighed into small vessels and the anaerobic glycolysis was measured by the usual manometric methods. During the 20-minute phase of obtaining temperature and pressure equilibrium, the CO_2 due to hydrolysis of the chloride was largely evolved, and afterward the fall in glycolytic rate was progressive with time; *e.g.,* during 1 hour the glycolytic rate fell 25-35 per cent; during 2 hours the glycolytic rate fell 50-60 per cent. When equivalent amounts of the other acid chlorides used in the animal experiments were substituted for myristyl chloride, the degree of inhibition of glycolysis was of the same order.

If, instead of the organic acid chlorides, an equivalent of HCl was similarly mixed with the tissue, the reduction of glycolysis was substantially the same; *e.g.,* when 0.314 N HCl was mixed with 5 gm. Jensen rat sarcoma tissue, the glycolytic rate fell, during half an hour, 35-40 per cent; and during an hour, 50-60 per cent.

The fact that the biochemical result is approximately the same whether acid chloride or HCl is minced with the tissue strongly suggests that simple hydrolysis predominates when the compound is in contact with living cells, and that there is little necessity to postulate a fixation of active groupings to account for their mode of action.

If an equivalent amount of a nonhydrolyzing chlor-compound; *e.g.,* chloracetone, be similarly minced with tumor tissue, the cessation of glycolysis is instantaneous, approaching 100 per cent. In this case the effective concentration greatly exceeds that necessary to study specific action, but its behavior stands in clear contrast with that of the hydrolyzing chlor-compounds.

Hence the concept of specific action on metabolic processes cannot be maintained for hydrolyzing chlor-compounds. They may be regarded as inflicting generalized damage to cells, markedly disturbing all cell processes, and in adequate amounts leading to cell death. The inhibition of glycolysis was here con-

veniently measured, but other reactions must also be depressed to an extent dependent upon their nature.

The contrast between the actions of nonhydrolyzing and hydrolyzing chlor-compounds is emphasized not only by their respective specific and nonspecific inhibition of glycolysis *in vitro* but also by their biological effect. The former type has been shown to affect the process of carcinogenesis in opposite directions according to the conditions of application; all the animal experiments so far carried out have shown that the latter type produce inhibitory effects only.

Discussion

The experiments recorded here bring to mind the anticarcinogenic action of mustard gas and its derivatives, as described by Berenblum (3).

To aid comparison of the two sets of results, the following brief summary of the facts presented in this and the two previous communications (5, 6) is made.

1. Nonhydrolyzing chlor-compounds (Soluble in fats and water)
   - *A. Effect on glycolysis*
     Check glycolysis reversibly. Degree of checking is proportional to the chemical reactivity of the chlorine atom.
   - *B. Effect on tumor induction.*
     (i) Inhibition, when applied in low concentrations over the same time periods as the carcinogen. This inhibition is annulled when higher concentrations are applied. Degree of inhibition is proportional to the chemical reactivity of the chlorine atom and therefore also to the degree of checking glycolysis.
     (ii) Stimulation, when applied after a preliminary but subeffective treatment with carcinogen.
2. Hydrolyzing chlor-compounds (Soluble in fats, insoluble in water)
   - *A. Effect on glycolysis*
     Checking is nonspecific, due to liberation of HCl. In testing the effect on metabolic processes, by manometric methods, the result is determined by the technic employed:
     (i) If mixed with minced tissue, metabolic rates fall as one of the consequences of generalized cell damage.
     (ii) If added to the saline medium, the much smaller decreases in metabolic rates are conditioned by the altered pH of the medium.
   - *B. Effect on tumor induction.*
     Inhibition, in all concentrations, however applied.

Berenblum and his colleagues (3, 4) attempted unsuccessfully to correlate the rate of hydrolysis, the inhibition of metabolic processes, and the anticarcinogenic action of mustard gas and some of its simple analogues and derivatives. The facts included in the above summary may suggest a reason for their failure. These workers made no distinction between hydrolyzing and nonhydrolyzing halogen compounds, thus comparing the biological effects of substances with essentially different properties. In view of these differences correlation is impossible.

Comparison of effects on metabolism.—In studying metabolic changes induced by mustard gas, etc., Berenblum *et al.* (4) employed 2 separate technics. The contrasting results obtained reflected the contrasting experimental methods of measurement, and not inherent differences in the properties of the compounds themselves. For example, they found that $\beta\beta'$-dichlorodiethylsulfide (mustard gas = M.G.) lowered the glycolytic rate considerably, while ethylene-bis-β-chloroethylsulfide (E.M.G.) had little effect on this process, though both compounds were rapidly hydrolyzed and were markedly anticarcinogenic. But M.G. was minced with the tissue and E.M.G. added to the suspension medium. Both substances belong to the class of hydrolyzing chlor-compounds, and, by analogy with the above-mentioned differential results obtained with a single acid chloride, the different technics would account for the apparent difference of action. By reversing the experiments; *i.e.,* mixing E.M.G. with minced tissue and adding M.G. to the suspension medium, it is highly probable that the apparent relative degrees of checking glycolysis would also be reversed.

These two sulfides, insoluble in water and easily hydrolyzed, cannot be compared in their effect on glycolysis with the 2 oxidation products of M.G.; *viz.,* $\beta\beta'$-dichlorodiethylsulfoxide and the corresponding sulfone, which are soluble in water and nonhydrolyzable. The sulfone is slowly hydrolyzed under physiological conditions of pH and temperature, but the effective checking of glycolysis *in vitro* is certainly due to the nonhydrolyzed compound. The latter fall into the category of chlor-compounds described previously (5), the varying degrees of checking glycolysis observed being a function of the different reactivities of their chlorine atoms. The sulfoxide appears to approximate in activity to α-chlor-diethyl ketone, and the sulfone to chloracetone, both as inhibitors of glycolysis and anticarcinogenic agents.

By thus dividing M.G. and its derivatives into 2 classes according to their solubility in water and hydrolyzability, they naturally fall in line with the many types of compounds, unrelated in general structure, which have been used in this work.

Comparison of effect on tumor induction.—The problem of the nature of the vesicant action of mustard gas is probably distinct from that of its anticarcinogenic action, since the dosage used in the latter case is small and its effect transitory. This view is also supported by the fact that the acid chlorides show no vesicant action and are innocuous in the concentrations used, in the sense that quick recovery follows any temporary damage to skin cells. Yet when used with

the chemical carcinogens their qualitative behavior is similar to that of mustard gas.

Though the inhibition of glycolysis *in vitro* by mustard gas can be explained entirely by its liberation of HCl, this may not entirely account for its anticarcinogenic action, though there seems no reason to postulate any action different, in this respect, from that of the acid chlorides.

An attempt to compare, from the available data, the relative anticarcinogenic potency of mustard gas and the acid chlorides is beset with difficulties. Berenblum (1, 2) used tar and 0.1 per cent mustard gas, and limited his experiments to a time when about 50 per cent of the control animals had developed tumors. There was a high mortality rate in his animals. He expressed his results in a way which precludes the calculation of an average induction time. For the experiments described in this paper 3,4-benzpyrene and 1 per cent acid chlorides were used. There was a low mortality rate, and the experiments were prolonged to 40 weeks; *i.e.,* more than twice the time necessary for 100 per cent of control mice to develop tumors. If the criteria used by Berenblum are applied to the experiments shown in Figs. 1 and 2, it will be seen that at a time when 50 per cent of the surviving control mice had developed papillomas, none had appeared in the mice treated with acid chlorides, except in the case of stearyl chloride, the least potent member used. By ending the experiment at arbitrary times any degree of inhibition may be claimed, from 100 per cent down to the significant percentage given by the average induction time.

It is clear that for a real comparison of relative effectiveness further experiments must be carried out under identical conditions. A similar qualitative action only is emphasized here. The common feature of all these hydrolyzing anticarcinogenic substances is their active halogen atoms. The nature of the molecular residue appears to be of secondary importance.

No doubt the number of compounds biologically active in the way described could be multiplied indefinitely. Apart from the active halogen atom, potency will vary with such physical characters as molecular size and the partition coefficient between fat and water. The toxicity of the compounds or their products of hydrolysis will also make its contribution to the net biological response.

Summary and Conclusions

The effect of a series of aliphatic, and one aromatic, acid chlorides on the rate of tumor induction in mice by 3,4-benzpyrene has been investigated. All proved inhibitory. The degree of inhibition varied with the molecular size and rate of hydrolysis, but did not run parallel with any single physical or chemical characteristic. The most effective was benzene sulfochloride, which hydrolyzes less rapidly than the aliphatic acid chlorides.

The action of acid chlorides on the glycolytic process of cells has been studied, and contrasted with that of the specific inhibitors of this process. It is concluded that the acid chlorides impair the activities of cells nonspecifically due to the liberation of HCl, and that the apparent inhibition of glycolysis is merely one expression of this generalized damage.

The work of Berenblum and his colleagues on mustard gas and allied compounds is examined in the light of the above results. The difference in biological action of hydrolyzing and nonhydrolyzing halogen compounds is emphasized.

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The Cocarcinogenic Action of Croton Resin

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In previous investigations on the role of irritation in carcinogenesis (1, 2), the author found that certain substances, when applied repeatedly to the skin together with a potent carcinogenic tar, had a pronounced inhibitory influence on tumor production. These agents were shown to be irritants, which owe their "anticarcinogenic" effect to some local influence on the skin, causing the latter to become refractory to the carcinogenic action of the tar, even when applied in subulcerative concentrations. The anticarcinogenic effect did not persist after discontinuing application of these agents, nor was any significant inhibition observed on the growth of tumors already established, unless ulcerative concentrations were used.

At the time, these results were interpreted as confirming some previous observations by the author, which tended to show that an optimal degree of irritation was necessary for carcinogenesis, an excess being as detrimental as an insufficient amount; for, when 2 carcinogenic agents, tar and freezing with carbon dioxide snow, were applied concurrently to the same area of skin, the induction of tumors was inhibited, but when each was applied separately at different periods, a summation of effect was obtained (3, 4).

It appeared, however, from subsequent experiments, that the 2 series of observations were not strictly comparable, since, unlike carbon dioxide snow, the anticarcinogenic agents mentioned above were not themselves carcinogenic (2), while of a large series of irritants tested in conjunction with a carcinogenic tar, only a small number showed any pronounced anticarcinogenic action (5).

The position appeared, therefore, somewhat confusing: some irritants were carcinogenic, and among these, either excessive or insufficient irritation was detrimental to tumor production; certain other irritants were not carcinogenic, but were capable of inhibiting the production of tumors; while others, again, were neither carcinogenic nor anticarcinogenic.

Was it possible that among the last named there were some which, without being themselves carcinogenic, could augment the tumor-producing action of a carcinogenic agent when the degree of irritation by the latter was insufficient? This might well be expected if irritation were in fact a necessary part of carcinogenesis.

The present investigations, begun in 1936, were originally undertaken to supply an answer to this question (6, 7).

Methods

The methods adopted were similar to those described in previous communications by the author, except that 3,4-benzpyrene was chosen as the standard carcinogenic agent instead of tar, and that the animals used were not of mixed stock from dealers, but consisted of a fairly homogeneous strain of white mice bred in this laboratory over a period of many years. The mortality rate during the course of the experiments was very low indeed (Table I, columns B and G), while the response to carcinogenic action under standard conditions was strikingly constant, the time taken for 50 per cent of survivors to develop tumors being 19½, 19, and 19 weeks respectively in 3 different groups of mice, tested at about 18-month intervals (Table I, series 1, 2, 3, column F).

The irritants used were dissolved in acetone in concentrations which, from previous determinations, were known to produce a moderate degree of irritation free from ulceration when applied at weekly intervals. The xylene, however, was used in an undiluted form. The solutions were applied to a small area of skin, about 1.5 cm. in diameter, in the region of the shoulder blades, after preliminary clipping of the hair with scissors. In studies on irritant action, it is, of course, essential to avoid the use of chemical depilatories. In some of the experiments (Table I, T, column A), the irritant and the carcinogen were applied together in a single solution once a week; in others (Table I, S, column A), they were applied separately at half-weekly intervals. In all cases, the frequency of application of any one agent was once a week.

The term "tumor" is used here to denote a papilloma at the site of application. No attempt was made to discover the incidence of malignant tumors, since the object of these investigations was the study of the influence of irritation on the first manifestation of neo-

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plastic transformation, which, in the case of the skin, represents the conversion of the preneoplastic thickened epithelium into a papilloma.

Four methods of indicating the rate of tumor production are given in Table I: (a) the total number of mice bearing tumors at the end of the experiment (column C), (b) the time taken for the first tumor to appear in the group (column E), (c) the time taken for 50 per cent of survivors to develop tumors (column F), and (d) the number and percentage of animals bearing tumors at the arbitrary period of 20 weeks from the commencement of the experiment (column G). Of these, the first is not a very accurate guide, since it is influenced by the duration of the experiment, and by the survival rate; the second is equally deceptive, since it merely indicates the response of 1 hypersusceptible animal, not of the group as a whole; the third and fourth are reliable methods, the third being a particularly trustworthy, yet simple, method of assessing carcinogenicity. However, in cases where the tumor yield is very low, and the 50 per cent level is never reached, one of the other methods is necessary for comparison.

TABLE I: SUMMARY OF RESULTS OF EXPERIMENTS SHOWING THE COCARCINOGENIC ACTION OF CROTON RESIN IN COMBINATION WITH 3,4-BENZPYRENE

		A	B	C	D	E	F	G	
Series		Mode of application †	Number of mice used	Total number of mice bearing tumors	Duration of experiment, in weeks	Time of appearance of first tumor, in weeks	Time taken for 50% of survivors to develop tumors, in weeks	Tumors/survivors. Percentage 20th week	
1	Control: 1% benzpyrene in acetone	T	36	24	21½	12	19½	22/36	61%
2	Control: 1% benzpyrene in acetone	T	36	25	22	11	19	18/34	53%
3	Control: 1% benzpyrene in acetone	T	40	30	21	5	19	26/39	66%
4	1% benzpyrene in acetone and 0.5% croton oil in acetone	S	36	27	21½	9	17	26/33	79%
5	1% benzpyrene and 30% turpentine	T	40	27	21	12	18	24/35	68%
6	1% benzpyrene in xylene	T	36	26	26	13	22	14/36	39%
7	Control: 0.05% benzpyrene in acetone	T	36	2	26½	21½	..	0/34	0%
8	Control: 0.05% benzpyrene in acetone	T	36	2	28	17	..	2/35	6½
9	Control: 0.05% benzpyrene in acetone	T	40	1	21	18	..	1/33	3%
10	0.05% benzpyrene in acetone and 0.5% croton oil in acetone	S	36	21	26½	11½	24	12/32	37%
11	0.05% benzpyrene and 0.5% croton oil in acetone	T	31	15	29	10	29	8/31	26%
12	0.05% benzpyrene in acetone and 30% turpentine in acetone	S	30	3	29	19½	..	1/30	3%
13	0.05% benzpyrene and 30% turpentine in acetone	T	40	0	21	..	..	0/38	0%
14	0.05% benzpyrene in xylene	T	36	2	28	17	..	2/25	8%
15	0.05% benzpyrene in acetone and 1% standard solution * of croton resin in acetone	S	20	16	20	9½	18	16/20	80%
16	0.05% benzpyrene in acetone and 1% resin-free croton oil in acetone	S	20	5	20	13½	..	5/19	26%
17	Control: 1% croton oil in acetone	T	60	1	27	16	..	1/52	2%
18	Control: 0.5% croton oil in acetone	T	36	0	24	..	..	0/34	0%
19	Control: 1% standard croton resin solution * in acetone	T	20	0	22	..	..	0/20	0%
20	Control: 30% turpentine in acetone	T	30	0	21	..	..	0/20	0%
21	Control: xylene alone	T	40	1	25	20	..	1/36	3%

* The standard solution contained 2.5 per cent crude croton resin in acetone, this being the concentration of the resin in croton oil. The 1 per cent standard solution (containing 0.025 per cent resin) corresponds, therefore, in resin content, to 1 per cent croton oil.

† T represents application in a single solution at weekly intervals. S represents application of the 2 solutions separately to the same area of skin, alternately at half-weekly intervals, each being, therefore, applied once a week.

RESULTS

Effect of turpentine, xylene, and croton oil on the carcinogenic action of 1 per cent benzpyrene in acetone.—Since the carcinogenicity of 1 per cent benzpyrene in acetone is very pronounced, the degree of irritation produced probably being almost optimal in intensity, any marked augmentation of carcinogenic action under such conditions could hardly be expected. Actually, a slight, but statistically insignificant, augmentation was observed with turpentine and croton oil.

An equally slight inhibition was found in the case of xylene, the 50 per cent level (Table I, column F) being 17, 18, and 22 weeks, when croton oil, turpentine, and xylene respectively, were used as the irritants in conjunction with the carcinogen, as compared to 19½, 19, and 19 weeks in the 3 controls (Table I, series 1-6).

Effect of turpentine, xylene, and croton oil on the carcinogenic action of 0.05 per cent benzpyrene in acetone.—Very different results were obtained when 0.05 per cent benzpyrene in acetone was used as the carcinogenic agent. In this concentration in the control experiments (Table I, series 7-9), only 5 of 112 mice developed any tumors at the site of application, the yield of tumors in the 3 separate experiments being 0.0, 6, and 3 per cent at the 20th week, while the 50 per cent level was never reached in any of these groups, although the applications were continued for 26½, 28, and 21 weeks, respectively. In fact, even the 10 per cent level was never reached.

The addition of turpentine or xylene produced no significant difference in the low yield of tumors (Table I, series 12-14). In contrast to this, the addition of croton oil, either applied together with the dilute benzpyrene solution (Table I, series 11) or separately (Table I, series 10), led to a pronounced augmentation of carcinogenicity, the yield of tumors at the 20th week being 26 and 37 per cent, and the 50 per cent level being reached after 29 and 24 weeks, respectively.

With this evidence of the existence of a "cocarcinogenic" action on the part of croton oil, and the fact that the other 2 irritants failed to elicit such a response, it was thought necessary to determine the fraction of croton oil which was responsible for this effect.

Effect of 2 separate fractions of croton oil on the carcinogenic action of 0.05 per cent benzpyrene in acetone.—Croton oil, a pharmaceutical product obtained from croton seeds, is a complex mixture of fatty substances, possessing toxicity, a powerful purgative action, and a marked irritant effect on the skin. The irritant principle, croton resin, can be separated from the remainder by differential extraction with petroleum ether from a solution of the oil in 90 per cent methyl alcohol. Additional details are given by Cherbuliez, Ehninger, and Bernhard (8).

As a first stage in the study of the constituents of croton oil responsible for the cocarcinogenic action, tests were carried out with the crude preparation of croton resin on the one hand, and the residual oil, after the resin had been completely extracted, on the other, the applications being made as before in conjunction with a 0.05 per cent solution of benzpyrene in acetone.

The result with croton resin showed that this product possesses an even more marked cocarcinogenic action than the total oil from which it was obtained (Table I, series 15 and 10), the yield of tumors at the 20th week being 80 per cent, as compared to 37 per cent with the total oil plus 0.05 per cent benzpyrene, and 0.0 to 6 per cent with 0.05 per cent benzpyrene alone.

With the resin-free oil, there was also some augmentation of carcinogenesis (Table I, series 16), though considerably less than with the croton resin; *i.e.*, 26 per cent, as compared to 80 per cent. In fact, in view of the small number of animals used in this test, the result with the resin-free oil may be less significant than would appear.

Tests for carcinogenic action of turpentine, xylene, croton oil, and croton resin.—Before attempting to assess the significance of the above results, it was necessary to determine if the irritants in question are themselves carcinogenic. Each irritant was, therefore, applied repeatedly to the mouse's skin under identical conditions to those of the above experiments, except that no benzpyrene was added to the treatment.

The results (Table I, series 17-21) indicate that the irritants are on the whole not themselves carcinogenic. No tumors were obtained with 0.5 per cent croton oil, 1 per cent croton resin or 30 per cent turpentine. On the other hand, 1 tumor appeared in a group of 60 mice treated with 1 per cent croton oil, and another in a group of 40 mice treated with xylene. Both these tumors were minute warts which never attained a size of more than 1 mm. in diameter, and both regressed and finally disappeared entirely.

Discussion

The augmentation of carcinogenicity by croton resin may be interpreted *a priori* in one of two ways: (a) the croton resin is itself a weak carcinogen and the combined action of croton resin and 0.05 per cent benzpyrene is due to a summation of effect on the part of 2 mild carcinogens, and (b) the effect of croton resin is specific, in the sense that the resin is able to augment carcinogenesis, without itself possessing any carcinogenic action.

The fact that 1 mouse did develop a tumor following repeated applications of croton oil in acetone, might at first sight be taken as evidence in favor of the first explanation. As against this it should be pointed out (a) that the "tumor" in question was a minute pedunculated wart which regressed after a short time, never to reappear, (b) that an occasional small wart of this kind, which never grows or becomes malignant,[1] is not infrequent with other apparently

[1] The one example of a malignant tumor arising among a group of 30 mice painted with commercial tetralin (9), is possibly due to impurities, since 100 mice painted with purified tetralin failed to yield any tumors.

noncarcinogenic irritants, such as xylene, as mentioned above and by Kennaway (9), oleic acid as noted by Twort and Fulton (11), and even the anticarcinogen, dichloroethylsulfide (2), and (c) that xylene failed to augment carcinogenesis, though it produced a tumor by itself.

There is, however, an even stronger reason for doubting that the cocarcinogenic action of croton oil or resin is dependent on its own slight carcinogenic potency. In the 3 controls with croton oil and resin (Table I, series 17-19), only 1 tumor developed in 116 mice, and for benzpyrene to produce so low a yield of tumors it would be necessary for it to be diluted to approximately 0.01 per cent. Therefore, if the cocarcinogenic effect of croton resin in conjunction with 0.05 per cent benzpyrene were dependent purely on the feeble carcinogenic action of the former, one should expect in the case of benzpyrene alone the tumor yield at the 20th week to be augmented from 0.0, 3, or 6 per cent to 80 per cent, by increasing the concentration of the benzpyrene from 0.05 to 0.06 per cent. It is inconceivable that so small a change in concentration could cause so marked an effect.

Hence, one must accept the alternative explanation that the cocarcinogenic action of croton resin is specific and that its augmentation of carcinogenicity is independent of its own doubtful carcinogenic action. The effect, moreover, is not unique, since a similar cocarcinogenic action has been described by Shear (10) in regard to a noncarcinogenic fraction of tar, though the constituent responsible in that case has not yet been determined.

Attention may be drawn to the following implications derived from the existence of cocarcinogenic action:

A. It has been pointed out (1) that the actual carcinogenic potency of a complex mixture such as tar may depend on a balance between the action of carcinogenic and anticarcinogenic constituents. A "noncarcinogenic" tar may conceivably contain a carcinogen, the action of which is masked by anticarcinogens present. The existence of cocarcinogens would seem to complicate the picture even further, and Shear (10) has demonstrated that such cocarcinogens are actually present in some tars. Their presence may possibly account for the difference in carcinogenic potency of tar and benzpyrene respectively, when tested on the rabbit's skin.

B. In clinical discussions on the relation of irritation to carcinogenesis, there is a tendency to oversimplify the issue by considering only 2 possibilities, either that all irritants are carcinogenic, or that irritation has nothing whatever to do with carcinogenesis. It is evident, however, that neither of these extreme views is tenable. Some irritants are undoubtedly carcinogenic, some are anticarcinogenic, others are cocarcinogenic, while the majority probably have no effect at all on the process of tumor induction. It is to be emphasized that the effect of irritation on the development of tumors already established is an entirely different problem from that of the effect of irritation on the process of carcinogenesis.

C. It is as yet impossible to estimate the extent to which cocarcinogenic action is responsible for an augmentation of tumor production in man. Until many hundreds of substances are tested for cocarcinogenic action, there is no means of telling whether it is a freak phenomenon or one which plays a significant part in the development of tumors. The fact that carcinogenic action in man is probably always sporadic and irregular and the intensity of action usually suboptimal, would suggest that ample scope may exist for cocarcinogenic action.

Summary

Croton oil causes a marked augmentation of carcinogenesis when applied at weekly intervals to the skin of mice in conjunction with a very dilute solution of benzpyrene in acetone.

Croton resin, a constituent of croton oil, possesses a similar cocarcinogenic action to an even more marked extent.

Two other irritants, turpentine and xylene, applied in concentrations which produce comparable degrees of irritation to that of croton oil, do not possess cocarcinogenic action when tested under identical conditions.

There is evidence that this cocarcinogenic action of croton resin is not dependent on any possible carcinogenic action which it may itself possess.

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Pre-excisional Fixation of Tissues in the Treatment of Cancer in Rats*

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A chemosurgical method for the removal of accessible neoplasms under complete microscopic control has been developed for use in human cancer. This is to be described elsewhere in the near future (2). The object of the present paper is to describe some of the animal research which led to the development of this method and which has been withheld from publication until the treatment had been applied clinically.

The basic idea was to devise a method by which cancers could be removed in successive layers, each of which could be studied systematically under the microscope to determine the presence or absence of malignant tissue. Such a technic would have the obvious advantage of enabling the removal of a given cancer with a minimal destruction of normal tissue.

The only practical and safe means of removing cancer tissues in layers required their chemical fixation *in situ* prior to excision. After such fixation incisions were made only through killed and fixed tissue thus avoiding all danger of spreading cancer cells over the cut surface. Moreover, the fixed, form-retaining specimens could be used for immediate frozen sections so that by systematic mapping of the area under suspicion in relation to anatomical landmarks, the exact location of residual areas of cancer could be determined. Further fixation was confined to the cancerous areas and the process was repeated until completely cancer-free tissue was reached.

Fixative preparations and method of testing.—The first necessary step was the selection of a satisfactory chemical fixative along with a suitable means for its administration. For purposes of testing, rats bearing transplanted Philadelphia I sarcoma, mammary sarcoma MS-2, Flexner-Jobling carcinoma, and carcinoma C-256 were used. Preliminary experiments showed that application of fixative solutions alone to the tumor surface resulted in insufficient penetration while intratumoral injection not only gave erratic penetration but was often fatal because of direct entrance of the medium into blood vessels. For deep penetration and accurate control the best procedure proved to be application of the fixative in the form of a paste.

The paste to be tested was applied to the tumor surface and covered with a cotton dressing held in place by adhesive tape which in turn was sewed to the skin to avoid loosening by teeth or claws. After 24 hours most of the fixed layer of tissue was excised. Since the incision was made through fixed tissue in which all vessels had been thrombosed no bleeding occurred unless the level of incision was inadvertently carried too deep. If bleeding was encountered it was promptly controlled by applying a small square of fixative-impregnated gauze under momentary pressure. If further action of the fixative was required another application of the paste was made to the surface exposed by the first excision.

A number of chemicals were tested not only for ability to produce satisfactory fixation for microscopic detail but also for toxicity, ability to diffuse into tissues, and controllability in regard to depth of penetration. Caustic alkalies, such as sodium or potassium hydroxide, produced liquefaction of tissues with complete loss of structure. Arsenic trioxide, phenol, and mercuric chloride were too toxic, while silver nitrate carried the danger of argyria. With antimony chloride the fixed tissues sometimes separated too rapidly making excision of layers of tissue difficult; moreover this chemical strongly affected normal skin requiring extraordinary care for its safe use. The most satisfactory fixative from every standpoint was zinc chloride, a chemical first extensively used in the treatment of cancer over a hundred years ago by Canquoin (1) and more recently in the treatment of inoperable breast cancer by Strobell (3). This substance in a proper base produced adequate fixation, penetrated well, was thoroughly controllable, produced no general toxicity, and was safe to handle since it has relatively little effect on the intact skin.

For accurate control of fixation over a wide range of depths it was found to be essential that the base carrying the fixative have certain special characteristics; namely, a low affinity for the fixative solution

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and a capillary structure. The low affinity for the fixative permits the chemical to leave the paste readily in favor of the tissues, while proper capillary structure enables the fixative to pass from all levels of even very thick applications, thus making it possible to vary penetration from less than 1 mm. to more than 1 cm. by simply altering the depth and area of application.

These properties may be obtained by certain combinations of "permeant" and "agglutinant" substances. The permeant is a finely granular material insoluble in the fixative solution and having a specific gravity of 4 or more; examples are pyrolusite, clinkers from bituminous coal, stibnite, limonite, hematite, ferric oxide, and ferric lactate. The agglutinant may be any of a variety of plant extracts such as *Sanguinaria canadensis, Phytolacca decandra, Podophyllum peltatum,* or *Inula helenium.* More common agglutinants such as starch or wheat flour are somewhat inferior but rye flour is fairly satisfactory. The agglutinant is used in just sufficient quantity to keep the fixative solution from separating from the base as it stands in the jar. The permeant supplies multiple interfaces making for a capillary structure; it also has low affinity for the fixative and along with the agglutinant gives body to the preparation.

The fixative is usually added in saturated solution. Zinc chloride should make up 40-50 per cent of the preparation by weight. A satisfactory preparation is the following:

Stibnite (80 mesh sieve)	40.0 gm.
Sanguinaria canadensis	10.0 gm.
Zinc chloride, saturated (4:1) solution	34.5 cc.

In this preparation (Z-108a) zinc chloride makes up 45.2 per cent of the weight.

Histology of tissues fixed in situ.—All of the fixatives tried with the exception of the strong acids and alkalies produced good enough fixation for easy diagnosis by one acquainted with the alterations in cell structure produced by each fixative. The dehydrating effect of zinc chloride caused some cell shrinkage with resulting increase in density of cytoplasm, pyknosis, and increased basophilism of nucleus and the appearance of clefts between the cells, but with experience the histological structure of the tissues could easily be recognized (Fig. 1). Antimony chloride caused the nuclei to become swollen and vesicular, and with too strong action, produced an amorphous basophilic mass useless for diagnosis. Phenol caused moderate shrinkage and distortion. Mercuric chloride and silver nitrate produced excellent fixation. The usual freezing or paraffin imbedding technics were used followed by hematoxylin-eosin staining.

In the normal tissues immediately surrounding an area of fixed tissue there develops after 24 hours a leukocytic infiltration which by 48 hours becomes a well defined wall of leukocytes. In this zone tissue dissolution occurs with resulting separation of the fixed tissue a few days later.

The leukocytic infiltration which is so prominent between fixed and unfixed normal tissues is almost entirely absent at the junction between fixed and unfixed malignant tissues. Because of this circumstance neoplastic tissues are rarely obscured by collections of leukocytes, hence diagnosis is easier.

Effect of chemical treatment on metastasis.—To test the possibility that the reactions set up in cancer tissue by treatment with fixative chemicals might increase the tendency to metastasis, repeated subcurative doses of zinc chloride were applied to a transplantable rat carcinoma. Flexner-Jobling carcinomas ranging in size from 2 to 4.5 cm. in diameter were paired off in equal sizes in the treated and control groups. In 4 experiments the treated tumors were injected every other day with 0.1 cc. of an 8-25 per cent zinc chloride solution, while the controls were untreated except for 1 group which received physiological saline solution. In 2 other experiments treatment consisted of daily applications of a small amount of a 40 per cent zinc chloride paste. Such subcurative doses were administered over a period of from 4 to 8 weeks. Rats dying during this period were examined for axillary, pulmonary, and mediastinal metastasis as were the rats killed at the termination of the experiment.

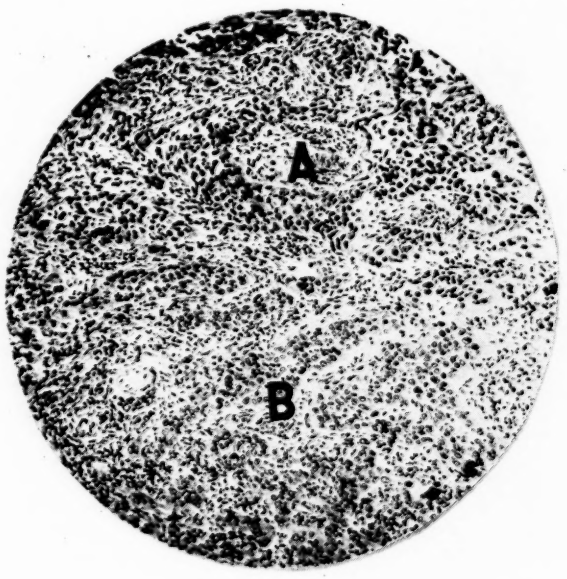

FIG. 1.—Flexner-Jobling rat carcinoma fixed *in situ* with zinc chloride in upper half (A) and *in vitro* with Bouin's solution in lower half (B).

The dehydrating effect of the zinc chloride is indicated by the cell shrinkage with resulting intercellular clefts and concentration of nuclear and cytoplasmic material.

A summary of the results of these experiments is given in Table 1. The failure of attempts purposely to increase metastasis by prolonged subcurative chemical treatment is evident. It indicates that certainly the rapid removal of tumors by chemical means could have no such tendency.

TABLE I: EFFECT OF REPEATED SUBCURATIVE DOSES OF ZINC CHLORIDE ON THE INCIDENCE OF METASTASIS OF FLEXNER-JOBLING CARCINOMA

	Treated animals			Control animals		
		Metastases			Metastases	
Treatment	No. rats	No.	Per cent	No. rats	No.	Per cent
Injection of 8 to 25% solutions	39	9	23.1	35	13	37.1
Application of 40% paste	22	8	36.3	18	9	50.0
Total, both methods	61	17	27.8	53	22	41.5

DISCUSSION

Because of the wide difference between the behavior of transplantable tumors and the available spontaneous neoplasms as they occur in animals, and the behavior of the usual accessible neoplasms encountered in humans, no serious attempt was made in these experiments to compare the efficacy of chemosurgical treatment with that of surgical or irradiation treatment. The expansive rather than invasive type of growth, the encapsulation, the easy visualization, and the lack of small caliber, unexpected outgrowths exhibited by the usual animal tumors, make them easily removable either by chemical or moderately wide surgical extirpation without the necessity of microscopic control. Comparison with other methods had best be made upon specific human neoplasms. This is being done in a clinical paper.

SUMMARY AND CONCLUSIONS

1. A technic for the pre-excisional fixation of cancer in rats is described. Fixation *in situ* is most controllable when the fixative is incorporated in a plastic base.

2. Of many fixative chemicals tested, zinc chloride proved to be the most satisfactory, and its incorporation in bases containing a combination of permeant and agglutinant materials resulted in preparations of low general toxicity, capable of accurate control over a wide range of depths of fixation.

3. The histology of tissues fixed *in situ* is modified in various ways by different fixatives. Zinc chloride causes slight shrinkage of cytoplasm and nucleus but the tissue loses none of its diagnostic value. Thus the progress of the removal of a neoplasm can be controlled microscopically, assuring complete extirpation with a minimum of normal tissue destruction.

4. Treatment of cancer in rats by fixative chemicals has no tendency to increase metastasis even when this is purposely attempted by prolonged subcurative dosage.

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The Urinary Excretion of Estrogens and Androgens by Women with Carcinoma of the Breast *†

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In view of the evidence in the literature that estrogens may play a role in the etiology of mammary carcinoma (4) while androgens may have an inhibiting effect (2, 5, 7), it seemed desirable to determine the concentrations of these sex hormones in the urines of the patients suffering from this disease. Because of the possibility that the factors which produce malignant changes in cells may occur early and disappear before the tumor is detected clinically the study of relatively young patients, in whom the disease might possibly be more acute, appeared to be most likely to give positive results. Therefore, patients who were still menstruating were selected.

Even such patients may have excreted large amounts of estrogens during the precancerous or early stages of the malignant tumor and may have ceased to do so at a later stage of the growth. For this reason, it was evident that failure to detect an increase in the urinary estrogen excretion would not rule out the hormone as an etiologic factor in human mammary cancer but would merely indicate that its excretion in abnormally high amounts is not necessary for the maintenance or progress of the disease.

Since very large doses of testosterone are necessary to inhibit cancer in mice, it seemed doubtful that even a marked reduction in the urinary excretion of androgens might be significant. However, female mice in parabiosis with males developed no mammary tumors although 50 per cent of the virgin females developed such tumors (6). The possibility that the continuous effect of small amounts of hormone might be as effective as the injection of massive doses at various intervals indicated the desirability of androgen assays.

Subjects and Methods

1. M. H. (New Haven Hospital No. A85659), white, female, age 49 years, married, was admitted on Nov. 27, 1938. Four years earlier she had had a simple right mastectomy. About 3 months before admission she discovered a small lump in the right axilla. Roentgen examination for metastases, Nov. 15, 1938, was indeterminate. A right radical mastectomy was performed, and the pathologic report was recurrent carcinoma of the breast with metastases to the axillary nodes. Postoperative irradiation to the breast was administered from Dec. 7 to Dec. 21, 1938. Twenty-four-hour specimens of urine were obtained daily from Dec. 10 to Dec. 22, 1938, upon which date the menstrual period began. A sterilizing dose of irradiation was given to the ovaries in January, 1939. When the patient was seen on Sept. 9, 1939, there was no evidence of metastases.

2. E. V. (New Haven Hospital No. 72058), white, female, age 45 years, unmarried, was admitted on Nov. 20, 1938. Two and one-half years before admission she had noticed a lump about the size of a walnut in the lower portion of her right breast. This had not increased appreciably in size and there were no associated symptoms. Physical examination revealed a hard mass 3 cm. in diameter, in the lower mid-line of the breast, with no change in the overlying skin except a slight dimpling at the lower margin of the tumor. The mass was fixed to the subcutaneous tissue but not to the skin. There were no palpable axillary nodes. On Nov. 21, 1938, a radical mastectomy was performed. The pathologic report was carcinoma of the breast with metastases to the axillary nodes. On Dec. 1, 1938, postoperative irradiation to the breast was instituted; irradiation of the ovaries was refused. Urine was collected in 24-hour specimens from Dec. 16 to Dec. 26, 1938. The menstrual period which was due at the latter date did not appear, but on Feb. 14, 1939, menstruation, normal in flow and in period of days, was resumed. On Jan. 19, 1940, there was no evidence of recurrent tumor.

3. M. N. (New Haven Hospital No. A77334), white, female, age 34 years, married, was admitted on Jan. 4, 1938. On Oct. 17, 1937, she had received a blow on the head which caused unconsciousness, followed by repeated headaches. Five weeks after the accident the patient noticed weakness and unsteadiness of the right hand, and on Dec. 15 weakness of the right leg was observed. On Jan. 4, 1938, she had a series of convulsions of the right side of the body. On Jan. 6 a tumor was removed from the left prefrontal region. The pathologic report was metastatic carcinoma. As there was a lump in the upper outer quadrant of the left breast, with fixation to the skin, and 2 large firm axillary nodes, a diagnosis of carcinoma of the left breast was made. A roentgen series on Jan. 24 revealed a metastatic tumor involving the ninth right rib posteriorly and possibly the eighth also. On Feb. 7, 1938, radium needles were inserted in the mammary tumor, which at first seemed to regress. On Dec. 10, 1938, however, a hard mass was palpated in the treated area and in the left axilla. There was also a firm mass along the anterior axillary fold and fullness in the left supraclavicular space. Twenty-four-hour urine specimens were obtained from Dec. 14 to 24. A menstrual period occurred from Dec. 17 to 20, inclusive, and the next began on Jan. 14. Death occurred shortly thereafter.

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† This paper is based upon a thesis submitted by Maurice Ross to the Faculty of the School of Medicine, Yale University, June 1940, in candidacy for the degree of Doctor of Medicine.

4. V. M. (New Haven Hospital No. A91955), white, female, age 45 years, unmarried, was admitted on Feb. 13, 1939. Five months before admission she noticed an intermittent bloody discharge from the right nipple. Two months before admission she discovered a lump in the right breast, which grew rapidly, ulcerating through the skin in about 1 month, after which it became tender and painful. The menses had been regular and there were no menopausal signs. The lower half of the breast showed a diffuse bluish induration and a large, firm, fixed mass. Roentgen examination of the skull, lungs, pelvis, and lumbar spine for metastases was indeterminate. On Feb. 14, 1939, a right radical mastectomy was done. The pathologic report was fibrosarcoma and carcinoma of the breast without axillary metastases. The menstrual period due just after the operation was limited to a slight spotting. Twenty-four-hour urine specimens were collected from Feb. 18 until discharge from the hospital on Feb. 23, 1939. Similar samples were collected from March 1 to March 11, 1939. On May 15 the patient was apparently in the best of health. She did not reappear until Oct. 16, 1939, when she had had a "cold" for one month and was spitting up blood "from the larynx." Roentgen examination on that day showed evidence of metastases to the lungs and mediastinum. Death was reported to have occurred on Dec. 31, 1939.

The urines were collected as 24-hour specimens, with toluene as a preservative, and were acidified by the addition of concentrated hydrochloric acid in a volume equal to one-tenth that of the urine. The specimens, usually for 3 consecutive days, but on one occasion for 2, were then pooled and boiled under a reflux condenser for 15 minutes. After cooling, the samples were extracted with benzene in continuous extractors (3). The crude benzene extract was evaporated under decreased pressure and the residue was dissolved in ether. The estrogens were separated by extracting the ethereal solution 5 times with 10 per cent sodium hydroxide. The alkali insoluble fraction containing the androgens was thus left in the ether layer. The ether was evaporated and the residue dissolved in 95 per cent ethanol.

The alkaline solution containing the estrogens was acidified with 16 per cent sulfuric acid and extracted 3 times with ether. This ether extract was evaporated to dryness and the residue was dissolved in 95 per cent ethanol. To transfer the androgens and estrogens to olive oil for the purpose of injecting into animals for bio-assay the proper quantities of the alcoholic solutions were evaporated over measured amounts of oil under decreased pressure. The estrogenic fractions were assayed on spayed albino mice by the vaginal smear technic of Allen and Doisy. Ten or more female mice were usually used for the assay of each of the preparations. The androgens were assayed by the comb-growth method in day-old female chicks (1). At least 10 chicks were used for each determination.

Results

The results as seen in Table I lie within the range of the urinary excretion of estrogenic and androgenic substances by normal women (3). The excretion of estrogens varied from <17 to 333 international units daily, which was in no case higher than that reported for normal females (3). The lowest values for the estrogenic substances were found in patients E. V. and V. M., who were not menstruating at the time of the collection of the urines. Menstrual periods reappeared in E. V. 2 months after the collection of the urines. Although the patient, V. M., had menstruated regularly up to the time of the urine collections, she died before menstruation was re-established. The lowest values for urinary excretion of androgens were 15 and 18 international units per day, in patient E. V., who was not menstruating at the time. Other values for the daily urinary output of androgenic substances varied from 20 to 40 international units and were, therefore, definitely in the normal range previously reported (3).

Table I: Urinary Excretion of Estrogenic and Androgenic Substances by Women with Carcinoma of the Breast

Patient and hospital number	No. of days urine combined	Days of menstrual cycle	Average daily estrogen excretion I.U.*	Average daily androgen excretion I.U.†
M. H.	3	17, 18, 19	167	40
A85659	3	20, 21, 22	83	..
	3	23, 24, 25	333	28
	3	26, 27, 28	63	..
E. V.	3	?	<17	18
72058	3	?	33	..
	3	?	33	15
M. N.	3	26, 27, 28	167	30
A77334	3	1, 2, 3	267	..
	2	4, 6	50	37
V. M.	3	?	<21	20
A91955	3	?	21	..
	3	?	100	27
	3	?	72	..

* The international unit (I.U.) of estrogenic activity is that evoked by 0.1 microgram of estrone.
† The international unit (I.U.) of androgenic activity is that evoked by 0.1 milligram of androsterone.

Summary and Conclusions

There is evidence in the literature to indicate that estrogens may play a role in the production of mammary carcinoma, while androgens may have an inhibitory action on such growths. In this investigation, the urines of 4 women suffering from cancer of the breast were assayed for androgens and estrogens, 3-day samples being used with 1 exception, when a 2-day sample was employed. Assays for estrogens were made on 3 of the samples from 2 of the patients, and on 4 from the other 2. Androgenic assays were performed on 2 samples from each patient. The quantities of urinary androgens and estrogens excreted did not vary appreciably from those reported for normal females.

These findings do not indicate that estrogens and androgens play no role in the etiology of mammary carcinoma; they merely demonstrate that the disease can be maintained or can progress in the presence of normal amounts of these hormones.

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The Effect of Estrogens and a Carcinogenic Chemical in Stimulating the Secretion of the Mammogenic Duct Growth Factor of the Anterior Pituitary*†

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When estrogens are injected into normal immature and castrated male or female animals growth of the duct system of the mammary gland is stimulated. During the past several years increasing evidence has accumulated indicating that this mammary hyperplasia is due not to a direct action of estrogen upon the mammary gland but instead to an increased rate of secretion of anterior pituitary hormones which have been called mammogen (17, 23, 18). Data recently published[1] indicate that this consists of 2 factors: a duct-growth factor and a lobule-alveolar-growth factor. A technic for the biological assay of the mammogenic duct-growth factor, in which young male mice are used, has been described (22). This method has now been extensively employed in assaying the mammogen content of pituitaries from dairy and beef cattle during several stages of growth, the sexual cycle, pregnancy, and lactation (23).

The object of the present paper is to describe how this same assay technic may be used to advantage in the direct determination of the ability of various estrogenic chemicals to stimulate the secretion of mammogen (duct factor) by the pituitary. Such information is not only of interest as indicating the relative effectiveness on mammary hyperplasia of various types of chemical compounds which have estrogenic effects, but has a far greater value in measuring the effect of carcinogenic chemicals upon the secretion of mammogen.

Lacassagne (21), Gardner, (13, 14), and others (1) have shown that the injection of estrogens into male mice of cancer-susceptible strains will induce the formation of mammary tumors. As mammary growth is a prerequisite for mammary tumor formation, it would appear that the direct cause of such tumors is the secretion of mammogen by the pituitary rather than the action of estrogens. It is conceivable that compounds having little or no estrogenic activity might so stimulate mammogen secretion as to lead eventually to mammary tumor formation. It would appear that in connection with the study of natural and synthetic carcinogenic compounds, the determination of their ability to stimulate the secretion of mammogen would be of great importance. The assay technic might also be applied to extracts of the blood and urine of patients having active breast tumors in order to determine whether there may be secreted unrecognized compounds which act upon the pituitary and cause increased secretion of mammogen.

Experimental

Procedure.—The method for the determination of the ability of various estrogens and carcinogenic chemicals to stimulate the secretion of mammogen by the anterior pituitary consists in the subcutaneous injection of the compound once daily for 6 days into male mice weighing 15 to 25 gm. The animals are sacrificed on the 7th day and the mammary rudiments examined for definite signs of duct development. A mouse unit is defined as the total amount of the chemical compound required per mouse to produce signs of duct growth in 1 or more glands of 50 ± 10 per cent of 10 or more male albino mice weighing 15 to 25 gm.

The chemicals assayed were dissolved in ether, if not already in solution, and were administered in 0.05 to 0.2 cc. sesame or olive oil except for the estriol which was in aqueous solution. Estradiol benzoate was obtained from the Schering Corporation, estrone and estriol from Parke, Davis & Company, diethylstilbestrol and triphenyl ethylene from E. R. Squibb & Sons, anol from the British Drug House, and 1,2,5,6-dibenzanthracine from the Eastman Kodak Co.

The mice were fed a mixed ground grain ration and Purina dog pellets *ad libitum*.

Results.—The 3 principal ovarian hormones were assayed in male mice in order to ascertain the dosage required when assayed for mammary hyperplasia under standard conditions. It was found that 200

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† Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 682.

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international units (20 γ) of estrone (theelin) were required for 1 mammogenic mouse unit (Table I), and 600 γ of estriol (aqueous theelol) gave a similar response. The estrone was therefore 30 times as effective, by weight, as estriol. The fact that the estriol was injected in water probably reduced its effectiveness compared with estrone in an oil carrier.

Surprisingly, estradiol benzoate proved to be many times more potent for mammary duct hyperplasia than estrone. The extremely small amount of 0.083 γ of the former gave a 41 per cent response in 17 mice. The most pertinent assay groups are given in Table I. The assay of estradiol benzoate actually involved 104 mice. This chemical appears to be 241 times as active per unit weight as is estrone for mammary duct growth.

On assay by the mammogenic mouse unit technic 0.05 γ of stilbestrol (4,4-dihydroxy α β diethyl stilbene) produced a 69 per cent response in 16 male mice. It is apparently at least 400 times as effective as estrone for mammogenic stimulation, and even more effective than estradiol benzoate.

Anol (p-hydroxyprophenyl), assayed by the mammogenic mouse unit technic, gave a 53 per cent response at 0.6 mgm. per mouse. Thus it appeared to be 1/30 as effective by weight as estrone and equal to aqueous estriol in promoting the production of mammogen in male mice and thus causing mammary hyperplasia.

Administration of triphenyl ethylene to 14 male mice at a total dosage of 0.5 mgm. caused a 36 per cent response, while 1.0 mgm. given to each of another group of 14 mice resulted in a 79 per cent response. One mammogenic mouse unit should, therefore, require about 0.7 mgm. Triphenyl ethylene is thus possibly 1/35th as effective as estrone and practically equal to aqueous estriol and to anol.

One mgm. of 1,2,5,6-dibenzanthracene administered to each of 9 male mice caused a positive mammary response in 56 per cent, indicating 1 mouse unit. This compound is possibly 1/75th as effective as estrone for mammogenic stimulation.

Table I: Comparison of Mammogenic and Estrogenic Activity of Several Estrogens and a Carcinogenic Compound

Preparation	No. of mice	Dosage in γ	Percentage response (positive)	Effectiveness (estrone = 1)	One estrogenic mouse or rat unit in γ	Reference
Estradiol benzoate (Progynon-B)	6	0.017	17	241 ×	0.1-0.25	(11, 20, 31)
	6	0.033	33			
	17	0.083	41			
	10	0.165	80			
4,4-dihydroxy α β diethyl stilbene (Stilbestrol)	10	0.2	60	At least	0.5-0.37	(26, 20, 9, 8)
	10	0.1	70	400		
	16	0.05	69			
Estrone (Theelin)	20	20	55	1	0.1-1.5	(11, 31, 20)
	6	10	33			
	16	0.25 to 2.5	0			
Estriol, aqueous (Theelol)	14	500	21	1/30	15.0	(31)
	19	600	42			
P-hydroxyprophenyl (Anol)	15	600	53	1/30	100-1,000	(26, 35, 15)
Triphenyl ethylene	14	500	36	1/35	1,000	(26)
	14	1,000	79			
1,2,5,6-Dibenzanthracene	9	1,500	56	1/75	0	(24, 5)
	13	2,000	77			

Discussion

In previous experiments, dosages of estrogenic compounds used to induce mammary hyperplasia have been based on comparative assays of genital potency. The present observations show that such evaluation is not of much advantage in studies on growth of the mammary gland and stimulation of mammogenic secretion in the anterior pituitary.

Some idea of comparative mammogenic potency could, however, be obtained on growth of the mammary glands. Turner *et al.* (32), for example, found that 2.5 rat units of theelin (urine extract) injected daily for 20 days into castrate female rats gave good response in growth of the mammary duct system. Similarly, 16.7 rat units of estrone daily for 12 days in male rabbits brought about considerable duct proliferation. Five rat units of theelin daily for 15 days produced no response in male mice, nor was any response obtained with 0.5 ml. of theelol (estriol). Gardner *et al.* (12), however, were able to produce well developed mammary glands in male mice with 2.0 rat units of estrone daily for 15 days. Turner and Gomez (33) found that 10 rat units of estrone injected daily for 20 to 30 days into adult male albino mice would

cause extensive growth of the duct system. At that time no standard assay technic for mammary growth was available, so that the comparative evaluation of various compounds from such experiments is difficult.

The comparative effectiveness of estrone and estriol observed in this study agrees rather well with the effect of the 2 estrogens on the vagina as determined by Thayer *et al.* (31). They assayed estrone and estriol by observation of the production of vaginal estrous changes in spayed female mice. Twenty times as much estriol was required for a 90 per cent response as of estrone for a 100 per cent response. For mammary growth, however, 40 times as much estrone or estriol was required as for genital stimulation.

Van Heuverswyn *et al.* (34) found that 16 γ of estradiol benzoate caused a ++ mammary response in 5 male mice when given in 8 injections over a 16-day period. A + response indicated an increased number of small, broader, branching ducts, usually terminating in enlarged end buds. Plus two glands were further developed.

Lewis and Turner (23) found that a daily dose of 3.3 γ of estradiol benzoate gave a good response (+, ++) in mammary duct stimulation in male mice when administered for 6, 8, and 10 days. In the present study it was found possible to decrease the total dosage to 0.083 γ per mouse and still obtain a 41 per cent response. This is a far greater potency, compared to estrone, than has previously been reported with comparisons on the basis of vaginal smears or uterine response. In the latter case estradiol was found to be only 3 to 5 times as active as estrone (31, 20). Our assay involves a large number of mice, however, and was characterized by consistent results. This dosage of 0.083 γ per mouse is very close to that required for genital response, whereas with most of the other estrogens tested the dosage required for minimum mammary response by this technic was considerably higher than that reported as necessary for genital response.

Folley and Watson (10) reported that stilbestrol produced changes in the chemical composition of cows' milk similar to changes produced by estrogen. Breast hypertrophy and genital changes were reported by Guldberg (19) in a castrate woman given 20.25 mgm. of diethyl-stilbestrol in 18 days followed by 30 rabbit units of progesterone in 6 days. MacBryde (25) reported similar results. Proliferation of the mammary epithelium was also revealed by biopsy specimens obtained before and after oral administration of 280 mgm. of stilbestrol to a castrate woman. No malignancy was apparent. One mgm. a day given orally for 14 days to another woman caused painful swelling of the breasts (Bishop, Boycott, and Zuckerman, 3).

Van Heuverswyn *et al.* (34) found that groups of 5 male mice given 0.05, 0.2, and 2.0 mgm. of stilbestrol, respectively, gave ++, +++, and + responses after 8 injections in 16 days. Stilbestrol was found to be about 1/5 as active as estrone in causing proliferation of the mammary glands of spayed rats (Felding and Möller-Christensen, 9) and of guinea pigs (Dodds, Lawson, and Noble, 6).

Assays of the effect of stilbestrol on the genital organs show that 0.5 γ produced estrus in 70 per cent of spayed rats weighing 175 gm (26). Stilbestrol was found to be equal to or 5 times as active as estrone in producing vaginal cornification in rats and mice (Morrell, 26; Emmens, 8; Sealy and Sondern, 29). Orally stilbestrol was 20 times as active in mice as estrone and 16 times as active as estradiol (Sealy and Sondern, 29).

In contrast to these reports, stilbestrol proved in our experiments to be at least 400 times as active as estrone in causing mammary proliferation in male mice. It was even more effective than estradiol benzoate. In view of this great activity, overdosage should be avoided in human therapy because of the danger of cancer promotion in susceptible individuals, as indicated by Auchincloss and Haagensen (2).

Anol, or p-hydroxyprophenyl, is a synthetic compound which has been found to have estrogenic activity of about 1 mouse unit per mgm. (26, 35). Dodds and Lawson (7) found that pure anol had no estrogenic properties, whereas the activity of the impure material was considerable. Bottomley and Folley (4) reported that anol stimulated growth of the nipples of guinea pigs. The condition of the mammary glands was not described.

Gomez and Turner (15) found that anol caused the production of mammogen in the pituitaries of rabbits. Thirteen mgm. of pituitary from young virgin does which had been given anol contained 1 mammogenic mouse unit, while pituitary tissue from untreated females and males was negative at 80 and 60 mgm., respectively. Anol plus estradiol appeared to be more effective than either alone.

Anol in amounts of 0.05 to 0.5 mgm. daily was reported by these same workers (16) to cause mammary duct development in male mice in 15 and 30 days. Female rabbits and rats also responded. Some abnormal development occurred.

With the assay technic anol was found to be fairly effective in causing stimulation of mammogen secretion and minimal signs of duct hyperplasia. Six-tenths mgm. was equivalent to 1 mammogenic mouse unit. This is 6 times the amount required for 1 estrogenic mouse unit of this material according to the assays made by Gomez (15).

Van Heuverswyn *et al.* (34) found that triphenyl ethylene administered to 3 groups of 7 to 10 male mice in doses of 5, 10, and 40 mgm. caused exten-

sive development of mammary rudiments (++ to ++++). Eight injections were given in 16 days.

Noble (27) reported that, although the estrogenic activity of triphenyl ethylene was low, it did have estrogenic properties as manifested by a lowered growth rate in rats and reduction of the gonadotropic activity of the pituitary following subcutaneous implantation of crystals.

Triphenyl ethylene was found to be fairly active in causing mammary duct hyperplasia in male mice. Interpolation of the 36 per cent response with a 0.5 mgm. dose, and 79 per cent response with a dose of 1 mgm. indicated that approximately 0.7 mgm. were required for 1 mouse unit response. This dose is within 0.1 mgm. of that required by estriol and anol.

Loeb (24) reported that 1,2,5,6-dibenzanthracene, which is actively carcinogenic (Shear, 30), is without estrogenic potency. Noble (27) found that crystals of 1,2,5,6-dibenzanthracene implanted subcutaneously did not reduce the growth rate of rats. The compound did not cause any decrease in pituitary gonadotropic activity, nor did it have estrogenic activity in male rats (Cook, *et al.* 5).

Perry and Ginzton (28) found that normal female mice from a low-tumor strain did not develop mammary cancer when 1,2,5,6-dibenzanthracene alone was applied cutaneously, but among spayed females similarly treated 4 per cent developed mammary cancer. When theelin was also given, tumors developed in 43 per cent, both of normal and spayed animals. This would indicate that 1,2,5,6-dibenzanthracene alone failed to produce tumors because of lack of mammary growth stimulation, which was supplied by estrone.

In spite of this lack of estrogenic and mammary carcinogenic properties, 1,2,5,6-dibenzanthracene was found by us to cause mammary duct hyperplasia in male mice. It was, however, the least potent of the compounds tested, 1.5 mgm. being required for a mouse unit response. The interesting point is that, though nonestrogenic, it will cause mammary growth. In this particular it compares with the mammogenic duct growth factor of the anterior pituitary.

Summary

Of various estrogens promoting mammary gland proliferation, the most active, as judged by the mammogenic mouse unit technic, was found to be stilbestrol, which proved to be at least 400 times as effective as estrone. Next in activity, among the compounds studied, was estradiol benzoate, which showed 240 times the effectiveness of estrone, by weight. Anol and triphenyl ethylene compared favorably with aqueous estriol but were only 1/30 as effective as estrone. 1,2,5,6-Dibenzanthracene was only 1/75 as effective as estrone.

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Experimentally Induced Benignancy of Neoplasm*†

V. The Influence of Hormones on the Host's Resistance to Implanted Neoplasm

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Several authors have reported the experimental production of neoplasms with estrogenic substances administered parenterally. These observations include not only the production of new growths in primary and secondary sex organs, but also of subcutaneous sarcoma by the implantation of crystalline estrone, as demonstrated by Gardner, Smith, Strong, and Allen (9).

Regarding the first group of observations, it is still debatable whether the hormone actually stimulates to malignancy, or merely hastens the development of tissue to the point where an independent predisposition to cancer may become effective. In the case of mammary tumor this point has been discussed by Lacassagne (15), by Geschickter (11), and by Nathanson and Andervont (18). In the case of the uterus, Gardner and Allen (10) classified seriatim the pathological stages which precede recognizable malignant tissue; *i.e.,* hyperplasia, secretory epithelial activity, stromal fibrosis, and pyometra. In this first group it is not clear that the action of the hormone is that of a true carcinogen.

In the second group, however, the female sex hormone appears to produce the same reaction as methylcholanthrene and other carcinogens, and these interesting demonstrations of carcinogenic effect might suggest at first sight that an excess of estrogenic hormone in the body always favors malignant growth. It is possible, however, to demonstrate another effect of the hormone, as reported from this laboratory in another publication (19).

This effect is not concerned with carcinogenesis, but rather with the fitness of the animal host as an animated culture medium for supporting the continued growth of a small island of tumor consisting of cells already clearly malignant. It has been demonstrated by Haddow and Robinson (12) and by Haddow, Scott, and Scott (13) that carcinogenic agents inhibit the growth of transplantable tumors and general body growth. These authors suspected from the work of Gardner *et al.* (9) that estrone might have a similar effect, but their experiments were negative. In our previous paper, however, it was brought out that such an effect could be demonstrated provided (a) the genetic constitution of the host were favorable, or (b) that the host's resistance against the neoplasm were increased by preliminary "immunization," either by live tumor cells, by non-viable tumor cells or even by tumor extract.[1]

Estrogens and immunity.—These experiments and the experiments reported in the present communication were performed with implanted sarcoma 180, in order to avoid confusion with the problem of carcinogenesis described above. The tumors were borne by 2 pure strains of mice; *i.e.,* black C57 and Bagg albino, strain A. It was found that in the black C57 strain some inhibition of a tumor growth did occur in mice treated with estrogens, as summarized in Table I. On the other hand, the albino animals showed no difference in the rate of growth when treated with estrone in huge doses. Nevertheless, when these albino animals were immunized first, the immunity was markedly enhanced, as shown in Table II (experiments 6a and 6b), which reveals a single but typical experiment. In brief, the control animals grew tumors in 98 per cent of cases, the immunized animals in 50 per cent of cases, and the hormone treated animals, following immunization, in no case.

When the immunity was not so efficient, some animals grew tumors as shown in Table II (experiments 7-9c), but even then the growth of these tumors was slow. Furthermore, these tumors behaved like benign

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[1] This "immunization" procedure is described elsewhere (20). Throughout this paper the term "immunity" is used in a specialized sense to indicate resistance against tumor growth on the part of the host.

tumors in many cases. For example, of 131 control animals, 89, or 68 per cent, were dead within a few weeks. On the other hand, after 5 months 76 per cent of 29 treated animals were living, and in some the tumors had regressed. This longevity of the treated animals emphasizes the effectiveness of the treatment.

TABLE I: INHIBITION OF TUMOR GROWTH BY ESTRONE

	Controls for virulence	Dose of estrone: 50 I.U. Group 1	Dose of estrone: 200 I.U. Group 2
Summary of experiments 1, 2, and 3 on black males, C57 / black females, C57			
Number of animals	46	12	50
Mean tumor diameter at 3 weeks, mm.	16.5	11.0	11.8
		100 I.U.	
Summary of experiments 4, 5, and 6 on albino males, strain A / albino females, strain A			
Number of animals	61	57	
Mean tumor diameter at 3 weeks, mm.	13.4	12.4	
Mean tumor diameter at 4 weeks, mm.	17.0	17.5	

TABLE II: ENHANCEMENT OF IMMUNITY IN DIFFERENT DEGREES BY ESTRONE

	Controls: Virulence	Controls: Immunity	Estrone treatment: Hormone alone	Estrone treatment: Hormone after immunization
	Experiments 6a and 6b combined			
Number of animals	52	16	..	28
Mean tumor diameter at 3½ weeks, mm.	19	9.1	..	0
Number of takes	51 *	8	..	0
Percentage of takes	98 *	50	..	0
	Experiments 7, 8, 9a, 9b, and 9c combined			
Number of animals	51	52	53	70
Mean tumor diameter at 4 weeks, mm.	20.0	13.1	19.0	8.7
Number of takes	49 *	31	53	21
Percentage of takes	96 *	60	100	30

* A second inoculation showed that susceptibility was really 100 per cent. In other words, the failure to take was due to a lapse in technic.

The following fact indicates that the inhibition is primarily an effect on the host. When small, slowly growing tumors, borne by immunized hosts, are reimplanted into untreated mice, the new growths prove highly malignant.

EXPERIMENTAL METHODS

A detailed description of the methods employed and dosage used in these studies of 632 animals is presented in another report, together with a statistical analysis of results and a more extensive survey of the pertinent literature (19). It should be noted that the use of mean diameter as a means of tumor size tends to minimize differences in tumor growth. The data summarized above would be more striking if tumor volumes had been calculated from the 3 diameters, as $V = 4/3\ \pi\ abc$, or by the prolate-spheroid formula ($4/3\ \pi\ ab^2$), as used by Emge and Murphy (6). The smaller tumors, furthermore, showed a low rate of mitotic activity, as reported elsewhere (3). Both mitotic counts in colchicine-treated tumors and counts obtained by direct microscopic study showed a significant correlation between mitotic activity and tumor size.

In summary, these large doses of estrone are of interest, first, because they picture estrogen as a motivator for benignancy, and, second, because they concern the suppression of a tumor made up of nondescript fibrous tissue unrelated to sex functions. The mechanism whereby this suppression is produced remains obscure, but certain features of the problem have already been explored, as follows:

Pituitary and thyroid.—In the Program of the Third International Cancer Congress, Ball, Samuels, and Schott (1) have discussed the influence of the hypophysis on the growth of malignant tumors in rats. Likewise, Cramer and Horning (4) have pointed out that the pituitary reacts to the growth of tumors in animals and that the adrenal often is involved. The effect of estrone observed by us might be explained, therefore, on the basis of a rearrangement of the balance between various endocrine glands of the animal host. On the other hand, it might conceivably be related more directly to the sterol structure of estrone. In order to test these possibilities we have continued our observations by noting the effect of anterior pituitary growth hormone, of thyroxine and of castration. These results are summarized below in Tables III, IV, and V and are seen to be essentially negative. Similar studies previously reported by Meyer, McTiernan, and Aub (16), but without preliminary immunization, were also negative. These data supplement studies on the adrenal by Ball and Samuels (2), who also have thus far been unable to explain the connection between the growth of neoplasms and the endocrine glands.

Testosterone.—The second possibility; namely, a peculiarity related to sterol structure, has also been tested in part by the use of other sterols. The results with testosterone propionate are summarized in Table VI and are negative, even though the dosage used was 0.25 mgm. of the propionate every other day for 1 month. Figures 1 and 2 indicate the degree of scatter in 1 such experiment.

Other authors have reported observations with testosterone, some of them positive. Flaks and Ber (7, 8), for example, have described an anticarcinogenic action

in mice painted with methylcholanthrene. Similarly, Duran-Reynals (5) showed that an aqueous extract of the testicles of rats and rabbits inhibited the transplantability and growth of the Brown-Pearce epithelioma; and Mu (17) produced increased susceptibility

cisely the reverse behavior to that of sarcoma 180 with regard to these sterols.

It is planned to test other sterols in order to explore further the effect of chemical structure in this respect. Investigations on progesterone are now in progress,

TABLE III: EFFECT OF ANTERIOR PITUITARY GROWTH HORMONE ON THE GROWTH OF MALIGNANT TUMORS

	Controls		Growth hormone *	
	Virulence	Immunity	Hormone alone	Hormone after immunization
Number of animals	12	8	15	7
Mean tumor diameter, 3½ weeks	16.5 mm.	9.5 mm.	14.6 mm.	11.2 mm.
Number of takes	12	4	15	4
Per cent takes	100%	50%	100%	57%
Average weight at start	25.0 gm.	22.0 gm.	26.3 gm.	22.8 gm.
Average weight at finish	29.2 gm.	24.0 gm.	30.0 gm.	27.0 gm.

* The material used was a preparation of Growth Complex No. 470, of Ayerst, McKenna, and Harrison, prepared from the anterior lobe of the pituitary of the hog. It contained 100 rat units (Collip) of the growth hormone, 10 rat units (Collip) of adrenotropic hormone, and an indeterminate amount of prolactin. Dosage: 0.5 rat unit (Collip) every other day for 4 weeks.

TABLE IV: EFFECT OF THYROXINE ON THE GROWTH OF MALIGNANT TUMORS

	Controls		Thyroxine *	
	Virulence	Immunity	Hormone alone	Hormone after immunization
Exp. 1				
Number of animals	31	30	.....	18
Mean tumor diameter, 3½ weeks	14.2 mm.	6.2 mm.	.....	5.3 mm.
Number of takes	31	18	.....	8
Per cent of takes	100%	60%	.....	45%
Exp. 2				
Number of animals	6	8	9	8
Mean tumor diameter, 2 weeks	11.7 mm.	7.2 mm.	10.3 mm.	9.8 mm.
Number of takes	6	6	9	6
Per cent of takes	100%	75%	100%	75%
Exp. 3				
Number of animals	5	9	15	20
Mean tumor diameter, 4 weeks	13.0 mm.	7.0 mm.	13.1 mm.	3.6 mm.
Number of takes	5	2	15	5
Per cent of takes	100%	22%	100%	25%

* Squibb's crystalline racemic thyroxine. Dosage: 0.005 mgm. thyroxine dissolved in alkali, by intramuscular injection every other day for 4 weeks.

TABLE V: EFFECT OF CASTRATION ON THE GROWTH OF MALIGNANT TUMORS

	Experiment	Controls C57		Castrated mice C57	
		Males	Females	Males	Females
Number of animals	1	6	6	12	12
Mean tumor diameter at 3 weeks, mm.	..	18.0	17.0	19.5	22.0
Mean tumor diameter at 4 weeks, mm.	..	21.0	19.0	25.0	27.0
Average deviation at 4 weeks, mm.	..	± 2.7	± 1.0	± 3.3	± 1.2
Number of animals	2	15	15	15	15
Mean tumor diameter at 3 weeks, mm.	..	20.1	14.0	18.5	18.0
Mean tumor diameter at 4 weeks, mm.	..	22.0	19.8	21.8	23.5
Average deviation at 4 weeks, mm.	..	± 1.7	± 3.8	± 2.2	± 2.8

of rabbits to Brown-Pearce epithelioma by administering estrogens, from human pregnancy urine, to male hosts. Heiman (14) obtained a like result with mammary fibro-adenoma in castrated rats injected with hormones. In short, the epithelial tumors showed pre-

but are not yet completed. Likewise, observations are being made with diethylstilbestrol, which is a powerful estrogen but not a sterol.

It should be emphasized that these data have nothing to do with carcinogenesis. Instead, they concern

the possibility of making the organism an unfit medium for the continued growth of malignant cells. The results thus far obtained do not distinguish between a strictly endocrinological effect and an inhibitory effect like that observed by Haddow (13). Even though the mechanism remains obscure, the results suggest that the fate of an implanted tumor can be determined in part by the endocrinological status of the host.

Summary and Conclusions

Several authors have reported the production of neoplasms with estrogen, administered parenterally. These interesting demonstrations of carcinogenic effect might suggest that an excess of estrogenic hormone in the animal body always favors malignant growth. It is possible, however, to show that estrone may inhibit the growth of implanted neoplasm. In this case, the sarcoma behaves as if benign in several characteristic

Table VI: Effect of Testosterone on the Growth of Malignant Tumors †

	Controls		Testosterone	
	Virulence	Immunity	Hormone alone	Tail treatment plus hormone
Exp. 1—Males				
Number of animals	8	11	14	15
Mean tumor diameter	15.8 mm.	11.5 mm.	16.9 mm.	11.0 mm.
Number of takes	8	7	14	4
Per cent of takes	100%	64%	100%	27%
Exp. 2—Males				
Number of animals	16	22	16	23
Mean tumor diameter	18.0 mm.	8.4 mm.	17.3 mm.	10.6 mm.
Number of takes	16	13	16	14
Per cent of takes	100%	59%	100%	61%
Exp. 1—Females				
Number of animals	12	20	10	17
Mean tumor diameter	11.8 mm.	5.2 mm.	13.2 mm.	8.5 mm.
Number of takes	10	6	10	3
Per cent of takes	84% *	30%	100%	17%
Exp. 2—Females				
Number of animals	17	23	16	23
Mean tumor diameter	17.7 mm.	10.1 mm.	16.1 mm.	6.8 mm.
Number of takes	17	7	16	7
Per cent of takes	100%	30%	100%	30%

† Dosage: 0.25 mgm. of testosterone propionate in oil, by intramuscular injection every other day for 4 weeks.

* Two reinoculations were successful, thus bringing the true percentage of takes to 100 per cent.

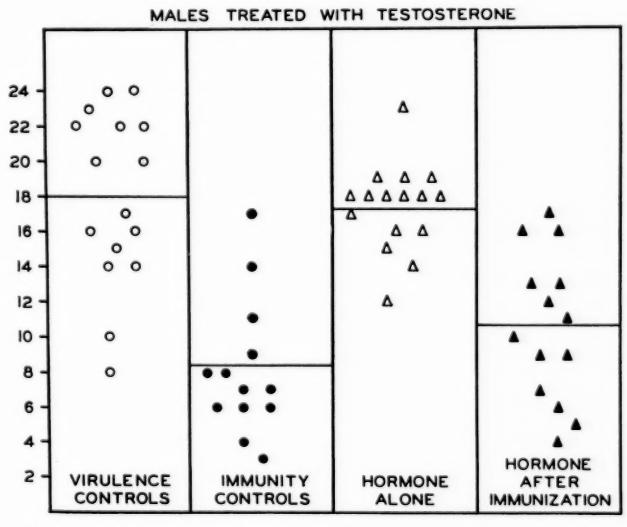

Fig. 1.—Testosterone in large doses failed to enhance the resistance of male mice against growth of sarcoma 180.

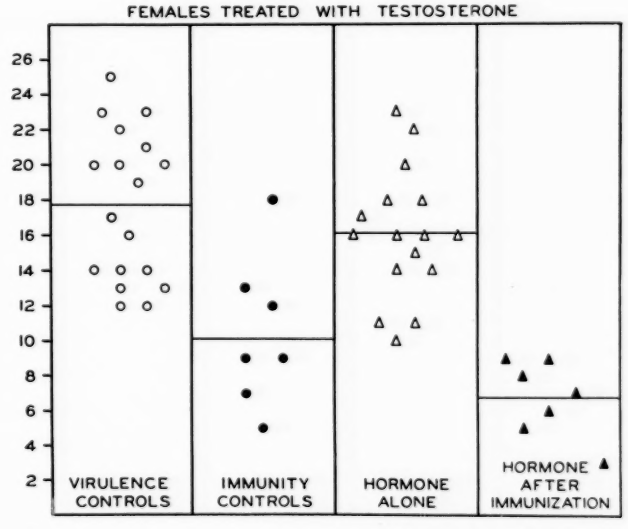

Fig. 2.—Testosterone in large doses failed to enhance the resistance of female mice against growth of sarcoma 180.

respects. This inhibition is probably not a direct inhibition of tumor tissue, but rather the enhancement of a primary resistance mechanism.

Homologous experiments performed with androgen, anterior pituitary growth hormone, and thyroxine were negative. Castration of females may decrease slightly the resistance to tumor growth, but the effect is slight at best.

These data have nothing to do with carcinogenesis, but rather concern the possibility of making the organism an unfit medium for the continued growth of malignant cells. They suggest that the fate of an implanted tumor is partly determined by the endocrinological status of the host.

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Spontaneous and Induced Tumors of the Guinea Pig

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The occurrence of tumors in the guinea pig is apparently less frequent than in other laboratory animals. Spontaneous tumors are quite rare and only a few transplantable tumors of the guinea pig are available. Jackson (22) in his monograph on the incidence of tumors of domesticated animals made no mention of guinea pig tumors.

One of the best known guinea pig tumors is Murray's (44) transplantable liposarcoma, non-metastasizing, which was primary in the mamma. Migunow (42) reported a non-metastasizing carcinoma of the breast in a male guinea pig. Two papillary cystadenomas of the breast were found by Apolant (3) and an adenocarcinoma of the breast by Sternberg (56) and one by Jones (23). Katase (24) mentioned a sweat gland carcinoma of the breast. A possibly induced, but probably spontaneous, tumor was the mammary adenoma studied by Anderson and Lumbroso (2) in a female guinea pig injected with urine which was being tested for the presence of tubercle bacilli. Blumensaat and Champy (8) found an apparent carcinoma of the right breast in a four year old female guinea pig with ovarian cysts. There were no metastases and grafts were not successful.

Giordano (17) reported a spontaneous teratoma in the ovary of a pregnant guinea pig. The Guérins (20) reported a malignant splenoma. Kröning and Wepler (28) found a spontaneous lipomyxofibroma of the psoas. Leader (30) reported an osteogenic sarcoma of the femur, and Roffo (47) mentioned a transplantable sarcoma. Brunschwig (9) reported a dermoid of the cornea. A teratoma of the pons was described by Lutz (39). Bablet and Bloch (5) reported a spontaneous tumor of the abdominal wall which they considered a schwannoma. There were no metastases and they made no attempts to transplant it. Maury's thesis (40) on tumors of the guinea pig is said to have emphasized their rarity, but it is not available to us.

A spindle cell sarcoma of the subcutaneous tissue of the back in a male guinea pig which metastasized to the spleen, and one of the subcutaneous tissue of the chest in a female which metastasized to lymph nodes, liver, spleen, lungs, ovaries, adrenals, and uterus were reported by Lubarsch (37). Both were transplantable. Bender (6) reported a round cell sarcoma of the heart of a guinea pig. Roskin (48) reported a malignant tumor of the adrenal of a guinea pig. Adenomas of the adrenal cortex developed after several years in guinea pigs castrated during the first days of life by Spiegel (53).

Two cases of adenoma of the bronchus were reported by Sternberg (55) and others by Spronck (54).

Loeb (36) reported 23 benign lesions of the ovary resembling chorio-epithelioma among 380 animals.

A probable lymphosarcoma was found by Miguez (41). Leukemia in the guinea pig was studied by Snijders (52).

The biliary tract of the guinea pig appears to be most susceptible to the development of induced malignancy. The earliest report of induced tumors of the guinea pig was that of Kazama (25). This report is not altogether clear as an unknown number of animals lived for only a short period of time. However, the frequency of development of tumors was very striking. When gall stones or pebbles were placed in the gall bladders of 30 guinea pigs, adenocarcinomas developed in 7 animals and metastases developed in 4. Stones placed in the stomachs of 20 guinea pigs produced no carcinomas. String sutures placed in the gall bladders of 23 guinea pigs produced adenocarcinomas in 6 animals, one of which metastasized; and mucosa placed in the gall bladders of 2 animals produced carcinomas in both, one of which metastasized. "Pityrol" injected into the stomachs of 7 guinea pigs and the urinary bladders of 7 guinea pigs produced no carcinomas, but when injected into the gall bladders of 16 guinea pigs it produced carcinomas in 8 animals. Lanolin injected into the urinary bladders of 3 animals produced no carcinomas, but when injected into the gall bladders of 27 it produced carcinomas in 6 animals. Tar injected into the gall bladders of 2 guinea pigs and into the urinary bladders of 2 others produced no carcinomas. This high percentage of successfully induced tumors has not been achieved by other workers.

In 1924 Kazama (26) reported that various types of irritants in the gall bladders of 244 guinea pigs produced 30 papillomas, 49 heterotopic growths, 60 atypical growths, and 101 adenocarcinomas of which 23 metastasized. Leitch (31) confirmed Kazama's results, producing 8 carcinomas of the gall bladder in 40 guinea pigs by implanting gall stones, pebbles, or pitch pellets. Delbet and Godard (13) produced in 6 guinea pigs, by the implantation of human gall stones within their gall bladders, 1 probable adenocarcinoma and, in a second series of 10 guinea pigs, 3 probable adenocarcinomas. They obtained no metastases and made no transplants. They reported that the injection of tar was without effect. The injection of irradiated ergosterol into the gall bladder by Schmid (50) produced tumors.

Radiation has produced tumors. One of 20 guinea pigs, 15 months after x-ray radiation, developed a polymorphocellular sarcoma of the back which did not metastasize and could not be transplanted (19). A chondrosarcoma of the tibia was produced by repeated x-ray radiation (38).

Another method of production of tumors by radiation was developed by Daels and Biltris (12). These experiments were broadened by Biltris (7) who produced, by the implantation of small amounts of radium element in collodion, a spindle cell sarcoma of the kidney, a spindle cell sarcoma of the meninges, and an intra-abdominal spindle cell sarcoma arising from the abdominal wall which was successfully transmitted by grafting; 6 carcinomas of the biliary tract, 1 of which produced metastases; and 2 tumors of the spleen. Gavrilov, Demoor, and Fester (15) found that no tumors could be produced by the injection of guinea pigs with the filtrate of the Daels-Biltris sarcoma and that repeated injection of the animals with embryonic guinea pig juice increased the number of induced tumors from 75 per cent to 90 per cent. Gavrilov and Silberfeld (16) reported that 11 of 74 guinea pigs injected with the Daels-Biltris tumor developed metastases.

Foulds (14) produced both carcinoma and sarcoma in guinea

pigs by injections of thorotrast. These tumors were transplantable and developed in 4 out of 9 guinea pigs given small injections of thorotrast in the breast tissue. Tumors comprised a carcinoma, 2 sarcomas, and a fibrosarcoma, of which only the first 3 were transplantable.

Petrov and Krotkina (46) placed radioactive glass in the gall bladders of 12 guinea pigs and produced metastasizing carcinoma in 2. They also placed glass without radioactivity in the gall bladders of 7 guinea pigs and produced metastasizing carcinoma in 2.

Lipschütz and others (33-35) have produced atypical uterine epithelium in guinea pigs by repeated injections of estradiol. Moricard and Cauchoix (43) produced large fibromas in the female guinea pig by injection of a benzoate of dihydrofolliculin. Nelson (45) reported in 1937 the production of fibromyomatous nodules in the uterus of guinea pigs by the prolonged administration of estrogenic hormones. While extreme growth of the cervical epithelium with metaplasia may be produced by administration of estrogens to the female guinea pig, Allen (1) found no instance of cervical carcinoma. Champy (10) produced adenomas and cysts in the ovaries of guinea pigs injected with folliculin.

The production of tumors in guinea pigs by injection of bacteria cultured from human carcinoma has been reported by Glover, and his views in this highly controversial field have been recently put forth (18).

Carcinogenic hydrocarbons have rarely been effective in producing tumors in guinea pigs. Instillation of tar into the bronchial tree produced adenocarcinoma of the bronchial epithelium (27). In 1936 Cirio (11) reported that he had painted guinea pigs with tar carcinogenic for mice and had obtained no tumors up to 2½ years. Latteri (29) succeeded in producing a tar cancer in the kidney of guinea pigs. Liberti (32) was unable to produce cancer with 1,2-benzpyrene (3,4-benzpyrene) in guinea pigs although it was carcinogenic for white mice and rats. A sarcoma of the heart, probably spontaneous, developed in 1 of 33 guinea pigs injected with methylcholanthrene (4). Liposarcomas were produced in 4 guinea pigs by 1,2-benzpyrene (3,4-benzpyrene) injected subcutaneously by Haagensen and Krehbiel (21), who also mentioned that only 2 spontaneous tumors of guinea pigs had been observed in 23 years at the Institute of Cancer Research of Columbia University. Painting the skin of guinea pigs with 1,2-benzpyrene (3,4-benzpyrene) failed to produce tumors (49).[1]

Shear (51), in collaboration with Drs. P. R. Howe and M. Elliott, injected 23 guinea pigs subcutaneously with 5 mgm. of crystalline 3,4-benzpyrene. Nineteen lived for 20 months, 1 developing a tumor during the 14th month which killed the animal in the 23rd month, and 1 developing a tumor at 20 months.

We have encountered only 1 spontaneous tumor in a guinea pig. The animal was received from a dealer in 1935 and proved to have a fibrosarcoma, probably of neurogenic origin, which had developed over the angle of the right jaw and which was ulcerated and partly necrotic. Attached to the maxilla and infiltrating the soft parts, it extended downward and mesially. X-ray of the bone was negative. The tumor, on removal, had a total volume of 4.5 cc. Centrally it was necrotic. On section, the viable portion was pale pink, fibrous, firm, and infiltrating the adjacent muscle. Transplants were made subcutaneously in the flank of 20 stock guinea pigs. Local tumors were obtained in 6 of the 20 animals. Transplants from one of these gave a subsequent tumor in 1 of 6 animals, and from another in 1 of 5 animals. Further transplants were unsuccessful. No metastases appeared in any animal.

We have found it difficult to induce tumors by carcinogenic hydrocarbons. In a series of 30 guinea pigs on a scorbutic diet (Howe-Wolbach), given just sufficient orange juice from time to time to prevent death from scurvy, 1 small nodule of actively proliferating granulomatous tissue appeared at the end of 142 days as a result of repeated injections subcutaneously of 1,2,5,6-dibenzanthracene (Eastman) dissolved in lard oil. This nodule we did not consider to be neoplastic. The other animals gave a completely negative reaction, as did the 6 similarly injected control animals on the same diet given an adequate amount of orange juice daily. Since in this series of experiments only a few of the animals lived beyond 100 days, the results were of little value.

No tumors developed in 12 normal guinea pigs which were followed for 15 months after subcutaneous injection with 5 mgm. of 1,2,5,6-dibenzanthracene in cholesterol pellets.

In a subsequent series of 44 normal guinea pigs woolen yarn which had been saturated with a benzene solution of 1,2,5,6-dibenzanthracene and allowed to dry was implanted subcutaneously. The dose was approximately 1 mgm. per animal. The animals were killed at intervals up to 4 months. No histologic evidence of tumor was obtained although there was marked focal necrosis and fibroblastic proliferation immediately surrounding the dibenzanthracene.

In a third series of experiments in which 5 mgm. of 3,4-benzpyrene (Hoffman-LaRoche, m. p. 178-179° C) suspended in 0.5 cc. of glycerine were given in a single injection subcutaneously, 53 guinea pigs lived over 196 days after inoculation (Table I), and 24 lived over 500 days.

Four tumors were obtained: a liposarcoma at 340 days, a fibrosarcoma at 454 days, a fibrosarcoma at 561 days, and a malignant mesenchymal tumor, probably a liposarcoma, at 579 days. This last tumor was noted when about 1 cm. in diameter, and killed the animal 53 days later, reaching a size of 4x5x5 cm. In none of the animals did metastasis occur. Death was apparently due to cachexia in all cases, probably secondary to the large size of the tumor and absorption from necrotic portions. Transplants were attempted from only 1 tumor, as the other 3 animals with tumor were found dead. From that tumor, a fibrosarcoma, no takes were obtained in 8 animals. Grafts of any tumor

[1] Reimann states he has rubbed with dibenzanthracene the skins of a number of guinea pigs three times a week for 5 years, the animals dying of old age without evidence of cancer. (Reimann, S. P. The Biology of the Cancer Cell. Am. J. Roentgenol. **43**:275-281. 1940.)

of the guinea pig rarely take as well as do those of transplantable tumors in mice, rats, or rabbits. Possibly better results would be obtained if guinea pigs of pure strain were used. These experiments were carried out on heterozygous animals.

TABLE I: RESULTS OF SUBCUTANEOUS INJECTION OF GUINEA PIGS WITH 3,4-BENZPYRENE SUSPENDED IN GLYCERINE

No tumor		Tumor		
Days after injection still alive	No. of animals	Days after injection tumor found	No. of animals	Type
196	11			
230	3			
289	1			
330	6			
		340	1	Liposarcoma
367	1			
371	1			
373	1			
395	1			
405	1			
425	1			
454	1	454	1	Fibrosarcoma
472	1			
519	3			
		561	1	Fibrosarcoma
		579	1	Liposarcoma (?)
589	6			
646	1			
654	9			
779	5			
	53		4	

SUMMARY

1. Cases of development of tumors, both spontaneous and induced, in guinea pigs have been collected.

2. One spontaneous and 4 induced tumors are reported.

3. The guinea pig appears to be resistant to the development of spontaneous tumors; to transplantation, at least in heterozygous animals, of tumors spontaneous and induced; and to the production of induced tumors other than of the gall bladder.

4. Carcinogenic hydrocarbons are much less potent in guinea pigs than in mice.

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Transplantable Lymphosarcoma of the Chicken

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EDITORIAL NOTE: On account of conditions caused by the war it has not been possible to exchange communications with Professor Pentimalli without long delays. Matters concerning his manuscript could not be taken up through the usual means of correspondence. In preference to an indefinite postponement of the publication of his observations the Committee has taken the liberty of issuing an abstract of his paper.

ABSTRACT

The tumor described in this paper arose in a Leghorn chicken selected for examination because of its pale comb and emaciation.

Examination of the blood revealed a severe change of erythroblastic type with the presence of numerous pathological elements difficult of classification. In nuclear structure the cells resembled at times lymphoblasts and at other times, hemocytoblasts. Nucleoli were present and the cytoplasm was strongly basophilic. Many of the cells were in mitosis. No granulocytes were observed.

At autopsy the liver was found to be enlarged, weighing 130 gm. It was covered with grayish white spots, measuring 0.1 to 2.5 cm. in diameter, separated by hepatic tissue of normal color. On section the organ appeared to be transformed into a mass of grayish white nodules of various sizes. On microscopic examination the normal hepatic tissue was found to be reduced to narrow bands separating the neoplastic nodules. The latter consisted of a loose connective tissue stroma containing accumulations of large round tumor cells with a nucleus rich in chromatin and showing nucleoli. The cytoplasm of some cells was scanty and of others, abundant. In some cells the chromatin was less profuse and was distributed in the form of a network, while the cytoplasm stained less deeply. Mitosis was observed in cells of both types. The arteries of the liver were intact, but the veins frequently showed destruction of their walls, and neoplastic tissue was present in the lumen at some points. In some places the small periportal veins were obliterated by compression. Although there were few blood vessels in the neoplasm itself necrosis was not observed.

The spleen measured 3.0 by 2.5 cm. in its greatest diameters. It had a uniform reddish brown color and did not contain nodules such as those present in the liver. The ovaries contained small whitish nodules. The femoral bone marrow was deep red in color and was removed with difficulty. There were no changes of significance in the lungs or other organs.

Smear preparations of the tumor tissue, stained by the May-Grünwald and Giemsa technic showed the 2 cell types even more clearly than microscopic sections.

Most of the cells were of variable size, containing a nucleus rich in homogenously distributed chromatin and 1 or 2 nucleoli. The cytoplasm, which was scanty or abundant, was intensely basophilic and free from granules. A halo of lighter cytoplasm was sometimes seen around the nucleus. Mitoses were numerous. These cells, with definitely pathological features, resembled lymphoblasts, but differed from them by their larger size.

The second less numerous type of cell was much larger, containing a coarse chromatin network, with clear spaces between the fibrils. The cytoplasm was much more abundant than that of the previously described first type of cells. It was slightly basophilic and contained no granules. Mitosis was frequent. These cells appeared to correspond to large atypical monoblasts.

The difference between these 2 types of cells lay in the formation of the nuclear chromatin. After transplants to the pectoral muscles of chickens the 2nd cell type, following intravenous injection of lithium carmine, was found to contain a single granule of the dye, while the cells of the first type showed no such inclusions.

The microscopic appearance of the kidneys was identical with those seen in the liver. The parenchyma was destroyed by compression and the tumor tissue had infiltrated between the renal tubules producing a picture characteristic of leukemic invasion. The small nodules in the ovary were composed of cells of the same type.

The normal structure of the spleen was so greatly altered that not even remnants of the follicles could be distinguished. The venous sinuses were barely visible. Erythroblastic cells of various degrees of maturity filled the sinuses. Hematocytoblasts, a few myelocytes, Rieder cells, and normal erythrocytes were also present.

The bone marrow was transformed into a firm tissue rich in immature erythrogenic and myelogenic cells. This tissue contained no evidence of blood vessels or sinuses. Some cell groups had characteristics similar

to those of the neoplastic tissue found in the liver, but no nodular formations were seen in the bone marrow.

Transplantation experiments.—Four chickens were inoculated in the pectoral muscle with an emulsion of hepatic tissue from the chicken bearing the original tumor. Five others were inoculated in the vein of the wing. Six chickens were inoculated intravenously with a protein preparation made according to a method previously described by the author. To prepare this material an extract of the liver was precipitated with 0.3 and 0.6 saturated solutions of ammonium sulfate, the precipitate was washed with ammonium sulfate, dissolved in a phosphate buffer solution at pH 7.4, precipitated, redissolved, and dialyzed.

No tumors developed in any of the chickens which had been injected intravenously with either the emulsion of the tumor or the solution of the precipitate from the extract. In the group of 4 chickens inoculated in the pectoral muscle with emulsion 1 tumor arose. This appeared 8 days after inoculation in the muscle. It measured 2 by 3 cm. and was soft in consistency. Histologically the cellular structure was identical with that of the original neoplasm. The muscular tissue was invaded by infiltration between the fibers. The 2 types of cells were present. Blood vessels were few, consisting of thin walls sometimes composed of a single layer of endothelial cells.

Four chickens were inoculated in the pectoral muscle with an emulsion of this transplanted tumor. Tumors developed at the site of inoculation and the neoplasm was carried through 23 transplant generations in a year. In each of these the characteristics of the primary lesion were reproduced. The tumor reached the size of a walnut in 1 or 2 weeks. It then infiltrated the entire pectoral muscle and caused the death of the fowl in 4 to 8 weeks.

The tumor rapidly acquired such virulence that by the 8th transplant generation practically 100 per cent takes were obtained. After the 8th transplant generation there was an apparent reduction of virulence, but this was largely regained later. A certain number of chickens, however, were resistant to the transplanted tumor.

The internal organs of animals bearing transplanted tumors were usually normal except for occasional slight enlargement of the spleen, which, however, was normal in appearance and consistency. Metastatic growths were seldom observed. Among 92 animals which developed tumors secondary deposits were found three times in the liver and kidneys, twice in the lungs and heart, and once in the spleen. Histological examinations of the organs probably would have shown more widespread and more numerous metastases.

In addition to the 5 chickens inoculated intravenously with an emulsion of the original tumor, 2 chickens were injected intravenously with an emulsion of the 4th transplant generation of the tumor, 6 with an emulsion of the 9th generation, and 4 with an emulsion of the 13th generation. None of these developed tumor in any organ.

The blood of animals receiving inoculations in the pectoral muscles, as well as of those injected intravenously, was examined at intervals of 6 or 7 days. Only transient changes were observed. These consisted of monocytosis without immature cells, and of anemia.

Filtration experiments.—The filterability of the tumor was tested at the 3rd, 8th, and 12th transplant generations. The results with Berkefeld V and N filtrates were negative.

Desiccation experiments.—Attempts were made to transmit the tumor with desiccated material from the 4th, 8th, 12th, 18th, and 20th transplant generations. All of these experiments gave negative results.

Resistance of the tumor to glycerine.—This was tested at the 20th generation by keeping the neoplastic tissue in glycerin for 8 days. The results were negative.

Discussion and Conclusion.—Professor Pentimalli reviews a number of papers by investigators who have studied transplantable tumors of the chicken and the leukoses of the fowl. The papers referred to are cited in the references. The editors have added several references to this list.

After discussing the characteristics of previously described tumors Professor Pentimalli concludes that the neoplasm described in this paper is the first example of transplantable lymphosarcoma of the chicken, and that it does not contain a filterable agent.

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Proceedings of the American Association for Cancer Research*

Thirty-Third Annual Meeting
Pittsburgh, Pa., March 19 and 20, 1940

MINUTES OF BUSINESS MEETINGS OF THE COUNCIL AND OF THE ASSOCIATION

A meeting of the Council was held at Hotel Schenley at 9:00 P.M. on March 18, 1940. Present: Drs. Andervont, Bagg, Clowes, Ewing, Little, Murphy, Simpson, G. M. Smith, Thibaudeau, Voegtlin, Woglom. Absent: Dr. Bell.

The minutes of the 1939 Council meetings were read and approved. The report of the Treasurer was accepted.

The following applicants were elected to membership in the Association: James S. Gallo, Haledon, N. J.; Richard Lewisohn, New York, N. Y.; Christ J. Christy, Buffalo, N. Y.; Earle B. Mahoney, Rochester, N. Y.; Wm. Bechmann Wartman, Cleveland, Ohio; Robert Hebell, Minneapolis, Minn.; Jesse P. Greenstein, Bethesda, Md.; Harold P. Morris, Bethesda, Md.; Mary E. Maver, Washington, D. C.; C. Donald Larsen, Bethesda, Md.; Helen I. Heiman, New York, N. Y.; J. A. Hoffman, Buffalo, N. Y.; Milton J. Eisen, New York, N. Y.; Howard H. Permar, Pittsburgh, Pa.; Lester Hollander, Pittsburgh, Pa.; George H. Fetterman, Pittsburgh, Pa.; Juan Z. Sta. Cruz, New York, N. Y.; Mortimer Cohen, Pittsburgh, Pa.; Theo. R. Helmbold, Pittsburgh, Pa.; Wm. Wright Pierce, Lockport, N. Y.; Harvey P. Barrett, Charlotte, N. C.; Dean Burk, New York, N. Y.; Morris K. Barrett, New York, N. Y.; Norman W. Elton, Buffalo, N. Y.; James B. Hamilton, New Haven, Conn.

The resignations of the following were accepted: Robert A. Lambert, New York, N. Y.; Moyer Fleischer, St. Louis, Mo.; B. S. Barringer, New York, N. Y.; Edmond M. Eberts, Montreal, Canada; H. W. Ingham, Jamestown, N. Y.

The Council expressed regret on the deaths of Mrs. George Semken, New York, N. Y.; Dr. Henry Schmitz, Chicago, Ill.; and Dr. Paul C. Weston, Jamestown, N. Y.

The proposed constitution and by-laws of the Association were discussed at length.

It was voted that the reports of the 1939 and 1940 meetings of the Association be sent to the American Journal of Cancer with the request that they be published in the near future.

It was voted that the Association hold its 1941 meeting in the same city and immediately before the meeting of the Federation of American Societies for Experimental Biology. (The next meeting of the Association will be held in Chicago, Ill. on April 15 and 16, 1941).

A nominating committee composed of Drs. Bagg, Murphy, and G. M. Smith was appointed to select the various groups of directors under the new constitution, which provides that the present Councillors divide themselves into groups of four serving one, two, and three years respectively. This committee selected by lot the following members of the Board of Directors to serve as follows:

For one year, 1940-1941: Drs. Bell, Clowes, Little, and Woglom.

For two years, 1940-1942: Drs. Ewing, Murphy, G. M. Smith, and Voegtlin.

For three years, 1940-1943: Drs. Andervont, Bagg, Simpson, and Thibaudeau.

The following officers were elected for the year 1940: *President,* Dr. Burton T. Simpson; *Vice-President,* Dr. George M. Smith; *Secretary-Treasurer,* Dr. A. A. Thibaudeau.

The meeting adjourned at 12:15 A.M.

A. A. THIBAUDEAU, *Secretary*

A business meeting of the Association was held at the Mellon Institute of Industrial Research at 9:25 A.M. on March 19, 1940. Dr. C. C. Little, President of the Association, called attention to the unusual importance of this meeting which would consider recommendations of the Council for incorporation of the Association and for the adoption of new by-laws.

Dr. Thibaudeau read the minutes of the meetings of the Council held during the past year.

Dr. Thibaudeau read the following certificate and articles of incorporation, as recommended by the Council after consultation with legal advisers:

* Publication of these Proceedings has been unavoidably delayed. In the interest of members of the Association *Cancer Research* takes pleasure in publishing the Proceedings in its first issue.

CERTIFICATE OF INCORPORATION

OF

AMERICAN ASSOCIATION FOR CANCER RESEARCH, INC.

PURSUANT TO THE MEMBERSHIP CORPORATIONS LAW OF NEW YORK STATE

We, the undersigned, all of whom are persons of full age, at least two-thirds of whom are citizens of the United States and at least one of whom is a resident of the State of New York, desiring to form a corporation pursuant to Section 10 of the Membership Corporations Law of the State of New York, do hereby make, sign and acknowledge this certificate as follows:

First: The name of the corporation is to be AMERICAN ASSOCIATION FOR CANCER RESEARCH, INC.

Second: The purposes for which the corporation is to be formed are to foster, support, promote, direct and conduct research in cancer, in diseases or conditions allied to cancer and in any and all other phenomena of cellular growth, by experimental, statistical, clinical, observational or any other means and through any of the natural, physical or social sciences, whether in their pure or applied phases, either directly or in cooperation with or through the medium of any other person, firm, corporation, association, foundation or institution.

Third: The territory in which the operations of the corporation are principally to be conducted is the United States of America and the Dominion of Canada.

Fourth: The principal office of the corporation is to be located in the City of Buffalo, County of Erie, State of New York.

Fifth: The number of directors of the corporation is to be not less than three nor more than fifteen.

Sixth: The names and places of residence of the persons to be the directors of the corporation until the first annual meeting of the corporation, are as follows: Dr. H. B. Andervont, 11 West Woodbine Street, Chevy Chase, Maryland; Dr. Halsey J. Bagg, Croton Lake, Westchester County, N. Y.; Dr. E. T. Bell, 526 Seventh Street S. E., Minneapolis, Minn.; Dr. George H. A. Clowes, Spring Hollow Road, Golden Hill, Indianapolis, Indiana; Dr. James Ewing, 415 Central Park West, New York, N. Y.; Dr. C. C. Little, Wayman Lane, Bar Harbor, Maine; Dr. James B. Murphy, 160 East 65th Street, New York, N. Y.; Dr. Burton T. Simpson, 108 Homer Avenue, Buffalo, N. Y.; Dr. George M. Smith, Pine Orchard, Connecticut; Dr. Alphonse Thibaudeau, 23 Irving Place, Buffalo, N. Y.; Dr. Carl Voegtlin, 2525 Belmont Road, N. W., Washington, D. C.; Dr. William H. Woglom, 12 First Street, Suffern, N. Y.

In Witness Whereof, we have made, signed and acknowledged this certificate.

After discussion, and on a motion duly seconded, it was:

Voted: that these articles of incorporation be and are approved.

The members of the Association considered the proposed by-laws which were read by Dr. Thibaudeau. In view of numerous questions and discussions it was decided to have mimeographed copies of the by-laws distributed to members of the Association during the day and to continue the consideration of them at a special business session to be held in the evening.

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The second business session of the Association was held at Hotel Schenley on the evening of March 19, 1940.

After the annual dinner, which was followed by a talk and moving picture demonstration entitled "Know Your Money," delivered by Mr. Haughton of the Secret Service, the business session was called to order at about 9:30 P.M. Dr. C. C. Little presided.

The chief items of business were consideration of the proposed new by-laws of the Association. Mimeographed copies of these by-laws as recommended by the Council had been distributed to all members at the morning session. President Little presented the by-laws article by article. The ensuing discussion was prolonged, and involved questions of policy, objectives, organization, and details of procedure and personnel. Numerous amendments, revisions, deletions, and additions to the draft of the proposed by-laws were adopted. Finally it was

Voted: to accept the by-laws as amended by the action of this meeting.

Dr. Little referred to the action taken at the morning business session at which on motion of Dr. Warren the members voted to adopt the articles of incorporation, as recommended by the Council. After further explanatory remarks by Dr. Little, it was

Voted: that the Association delegate to its President, Secretary, and such other members of the Council as may be necessary the execution and filing of the certificate of incorporation and of the by-laws as approved at this meeting, subject to such minor changes of detail and not of meaning as may be necessary to conform with the law.

A recess was taken at 10:55 P.M. The meeting was called to order again at 11:00 P.M.

President Little presented the question as to whether the Association should meet in the future with the Federation of American Societies for Experimental Biology, or the American Association of Pathologists

and Bacteriologists, or hold a separate meeting of its own. He reported that the Council had been almost evenly divided on this question, and had voted, 5 to 4, to try affiliation with the Federation of American Societies for Experimental Biology for a period of two years.

After considerable discussion President Little asked for a show of hands on these questions.

The meeting adjourned at 11:30 P.M.

(Abstract of transcript by S. Bayne-Jones)

A meeting of the Council was held at Hotel Schenley at 12:15 P.M. on March 20, 1940.

Present: Drs. Andervont, Bagg, Clowes, Ewing, Little, Simpson, G. M. Smith, Thibaudeau, Voegtlin, and Woglom. Absent: Dr. Bell.

Dr. G. M. Smith was appointed Chairman of the Program Committee.

A Standing Committee of three to deal with matters concerning the Journal was appointed as follows: Dr. Little, chairman, Dr. Murphy, and Dr. Woglom.

There was some discussion of the character of papers to be presented at the annual meetings.

The following nominating committee was appointed: Dr. Bagg, chairman, Dr. G. M. Smith, and Dr. Voegtlin. This committee was directed to furnish a list of eight members whose names should be placed on the ballot for the election of four new directors for 1941. These ballots are to be sent by the Secretary to all members before the Annual Meeting in 1941, with space for additional names which members may wish to place in nomination.

The articles of incorporation were signed by the directors.

The meeting adjourned at 1:15 P.M.

A. A. Thibaudeau, *Secretary*

SCIENTIFIC SESSIONS

Editorial note.—It is regretted that there has not been time since the establishment of this journal to obtain abstracts of the papers presented or to prepare and circulate for approval abstracts of the discussions. In order to make a permanent record of the subjects considered the program is reprinted, as follows:

Tuesday Morning Session—March 19, 1940
Mellon Institute for Industrial Research
Dr. C. C. Little, Presiding

Address of the President: Dr. C. C. Little, Bar Harbor, Maine.

Wm. Carpenter MacCarty, Sr., Rochester, Minn.: The Carcinogenic State.

Warren H. Lewis, Baltimore, Md.: Nucleoli of Normal and Malignant Cells.

E. von Haam and L. Cappel (by invitation), Columbus, Ohio: The Influence of Hormones upon Cells grown in Tissue Culture.

R. J. Behan, Pittsburgh, Pa.: The Value of Cancer Research in Clinical Procedures.

Harold A. Solomon and E. M. Burke, Buffalo, N. Y.: Malocclusion and Alveolar Resorption as Etiologic Factors in the Production of Oral Tumors.

E. M. Burke, Buffalo, N. Y.: Metastatic Tumors of the Spleen.

Tuesday Afternoon Session—March 19, 1940
Mellon Institute for Industrial Research
Dr. G. H. A. Clowes, Presiding

Joseph H. Farrow, New York, N. Y.: The Treatment of Inoperable Carcinoma of the Breast with Testosterone Propionate.

Shields Warren, Boston, Mass.: Skin Cancer in Relation to Multiple Malignancy.

Wm. C. Hueper, Scarsdale, N. Y.: Rhabdomyomatosis of the Heart in a Guinea Pig.

Ira T. Nathanson, Boston, Mass.: Studies on the Etiology and Treatment of Leukoplakia Buccalis.

Stanley G. Warner, M. C. Reinhard, and H. L. Goltz, Buffalo, N. Y.: Some Effects of Light Doses of X-ray on the Transplantability of a Mammary Tumor.

W. E. Heston, Bar Harbor, Maine: The Susceptibility of Four Inbred Strains of Mice and their Hybrids to Induced Pulmonary Tumors.

H. B. Andervont, Bethesda, Md.: Susceptibility of Hybrid Mice to Dibenzanthracene Induced Tumors.

Elizabeth B. Jackson and Austin M. Brues, Boston, Mass.: Studies on a Transplantable Embryoma in Mice.

Hugh J. Creech (by invitation) and R. Norman Jones (by invitation), Cambridge, Mass.: The Conjugation of Carcinogenic Hydrocarbons with Amino Acids and Proteins.

Wm. S. Murray and Joseph Hoffman, Buffalo, N. Y.: The Method of Measuring the Incidence of Cancer in a Population of Mice.

Wm. S. Murray, Buffalo, N. Y.: Studies on the Inheritance of Mammary Cancer in the Mouse.

R. Lewisohn, R. Leuchtenberger (by invitation) and D. Laszlo (by invitation), New York, N. Y.: The Treatment of Spontaneous Breast Tumors in Mice.

Wednesday Morning Session—March 20, 1940
Foster Memorial Hall, University of Pittsburgh
Dr. C. C. Little, Presiding

Wm. J. Hoffman, New York, N. Y.: Hormone Therapy of Male Breast Hypertrophy.

Jacob Heiman, New York, N. Y.: Hormonal Studies in Relation to Tumor Growth.

Harold P. Morris and Carl Voegtlin, Bethesda, Md.: Effect of Methionine on Normal and Tumor Growth.

Egon Lorenz and H. B. Andervont, Bethesda, Md.: Incidence of Induced Lung Tumors in Susceptible Mice Raised in Dust-free Atmosphere.

Egon Lorenz and Harold L Stewart, Bethesda, Md.: Experimental Intestinal Carcinoma in Mice.

Harold P. Rusch, Madison, Wis.: A Study on Tumor Production with Ultraviolet Irradiation.

M. J. Shear and Harold L. Stewart, Bethesda, Md.: Carcinogenic Activity of Nitrogen-containing Benzene Derivatives.

M. B. Shimkin and Hugh A. Grady, Bethesda, Md.: Carcinogenicity of Stilbestrol and Estrone.

Julius White, Bethesda, Md.: Effect of Oral Administration of Butter Yellow on the Growth of the Rat and the Effect of Various Supplements.

D. Shemin (by invitation), E. E. Sproul, and J. W. Jobling, New York, N. Y.: The Transmissible Agent of the Rous Chicken Sarcoma No. 1.

Wednesday Afternoon Session—March 20, 1940
Foster Memorial Hall, University of Pittsburgh
Dr. C. C. Little, Presiding

Leonell C. Strong, New Haven, Conn.: Genetic Analysis of the Induction of Tumors by Methylcholanthrene.

Kanematsu Sugiura, New York, N. Y.: Experimental Liver Cancer in Rats and its Inhibition by Rice Bran Extract.

A. M. Cloudman, Bar Harbor, Maine: Pseudo Hybridization, a New Type of Maternal (Embryonic) Influence upon Mice arising from Transferred Ova.

Fritz Bischoff, M. Louisa Long (by invitation) and J. Jerome Rupp (by invitation), Santa Barbara, Cal.: Influence of Induced Hibernation on the Growth Behavior of Mouse Neoplasms.

M. E. Krahl, Miss A. L. Keltch (by invitation) and G. H. A. Clowes, Indianapolis, Ind.: Metabolism of Tumor Tissues as a Function of Temperature.

G. H. A. Clowes, K. K. Chen (by invitation), Paul N. Harris and C. L. Rose (by invitation), Indianapolis, Ind.: The Effect of Reduced Temperature on the Development of Primary Breast Tumors in Mice.

Paul N. Harris, Indianapolis, Ind.: Induction of Sarcoma by the Injection of Wheat Germ Oil.

J. J. Bittner, Bar Harbor, Maine: The Role of Genetics in Breast Cancer Studies in Mice.

Florence S. Jenney (by invitation), Pittsburgh, Pa.: Transplantation of a Reticulum Cell Lymphosarcoma of the Rat through Twelve Generations.

Earle B. Mahoney (by invitation) and J. J. Morton, Rochester, N. Y.: The Neoplastic Response of the dba (Little) strain Mice to Methylcholanthrene.

George O. Gey, Baltimore, Md.: The in vitro Production of a Transplantable Sarcoma from a Strain of Normal Rat Fibroblasts maintained in Continuous Cultures.

Abstracts

Abstracts are grouped under the following main headings: Reports of Experimental Research, Comparative Oncology, Clinical and Pathological Reports, Statistics, and Cancer Control and Public Health. Subheadings are used in accordance with the subjects of papers abstracted.

The initials of the abstractor are placed at the end of each abstract. Contributors of abstracts published in this issue are: S. Bayne-Jones, E. Boyland, A. Haddow, E. L. Kennaway, A. A. Liebow, L. L. Waters, and H. Gideon Wells. It is a particular pleasure for Cancer Research to acknowledge the receipt of abstracts from Professor E. L. Kennaway and his associates. Professor Kennaway and Dr. W. E. Gye have kindly agreed to arrange for the abstracting of articles appearing in publications in the British Commonwealth.

REPORTS OF EXPERIMENTAL RESEARCH

Carcinogenic Compounds

BERENBLUM, I. (Oxford Univ. Research Centre of the British Emp. Cancer Campaign, Sir William Dunn Sch. of Path., Univ. of Oxford, Oxford, England.) The cocarcinogenic action of croton resin. Cancer Research, 1:44-48. 1941.

Croton oil and croton resin, in various concentrations, were applied to the skin of mice in conjunction with painting with different amounts of 3,4-benzpyrene. Both the oil and the resin augmented the carcinogenic action of benzpyrene. The cocarcinogenic action of croton resin was stronger than that of the total oil from which it was derived. Turpentine and xylene applied in concentrations which produce comparable degrees of irritation to that of croton oil did not have cocarcinogenic action. The mechanism of cocarcinogenic action is discussed.—S. B-J.

COOK, J. W., and R. H. MARTIN. (The Chester Beatty Research Inst., The Roy. Cancer Hospital (Free), London, England.) Polycyclic aromatic hydrocarbons. Part XXIV. J. Chem. Soc., 1125-1127. 1940.

Photo-oxides of several homologues of 1,2-benzanthracene with substituents at positions 9 or 10 or both (namely 9-methyl-, 10-methyl-, 10-isopropyl-, 9,10-dimethyl-, 5,9,10-trimethyl, 6,9,10-trimethyl-, and 5,6,9,10-tetramethyl-1,2-benzanthracene), and of 9,10-dimethyl-1,2,5,6-dibenzanthracene, were prepared by passing oxygen through solutions of the hydrocarbons in CS_2 during exposure to light. The structure of the photo-oxide of 9,10-dimethyl-1,2-benzanthracene was established as that of a peroxide involving both meso-carbon atoms. Derivatives of 1,2-benzanthracene with only one meso- substituent (9-methyl-, 10-methyl, 10-isopropyl) gave photo-oxides less readily. The capacity to form these compounds appears to be unrelated to carcinogenic activity. Tumors were not produced by injection *sub cutem* in mice of the photo-oxide of the highly carcinogenic 9,10-dimethyl-1,2-benzanthracene, and it has not been possible to isolate photo-oxides from 1,2,5,6-dibenzanthracene, 3,4-benzpyrene, or 1,2-dimethylchrysene.—E. L. K.

COOK, J. W., and E. L. KENNAWAY. (Roy. Cancer Hosp. (Free), London, England and the Univ. of Glasgow, Glasgow, Scotland.) Chemical compounds as carcinogenic agents. Second supplementary report: Literature of 1938 and 1939. Am. J. Cancer, 39:381-428 and 521-582. 1940.

With the addition of this review article to those appearing in the Am. J. Cancer, **29**:219. 1937 and Am. J. Cancer, **33**:50. 1938, the vast literature on chemical compounds as carcinogenic agents from 1933 to 1939 becomes available in summarized form. These reviews with their appended bibliographies of 860 titles are invaluable to anyone engaged in experimental cancer research.—L. L. W.

CRABTREE, H. G. (Imperial Cancer Research Fund, Mill Hill, London, England.) Influence of solvent on the rate of induction of epithelial skin tumours in mice. J. Path. & Bact., 51:299. 1940.

The absence of any uniformity of technic makes comparison of the results of different workers very uncertain. The following results show the importance of one factor, namely solvent.

Effect of solvent on the carcinogenic action of 3,4-benzpyrene (0.3% applied twice weekly to 40 mice in each series)

Solvent	Percentage of mice bearing tumors	Average induction time for papillomas (weeks)
Ether + 2% liquid paraffin	97.5	12.1
Ether alone	95.0	14.3
Benzene + 2% liquid paraffin	100.0	14.9
Benzene alone	92.5	18.0

Liquid paraffin, which was added to retain the carcinogen in a film at the site of application, thus accelerates the action with either solvent by about the same amount. A similar experiment showed that 1,2,5,6-dibenzanthracene in acetone + 2% liquid paraffin acted much more rapidly than in benzene.—E. L. K.

DES LIGNERIS, M. J. A. (South African Inst. for Med. Research, Johannesburg.) The production of benign and malignant skin tumours in mice painted with Bantu liver extracts. Am. J. Cancer, 39:489-495. 1940.

It is an established fact that the Malayan natives of the Dutch East Indies and the Bantus of South Africa show a high incidence of primary liver cancer, while the Europeans in these countries do not. This paper describes an attempt to extract from the livers of Bantus a possible causative chemical agent. Carcinomatous and noncarcinomatous liver tissue from Bantus was used as experimental material, and livers from noncancerous Europeans served as controls. The extraction process consisted of preliminary digestion with strong alkali in alcoholic solution, and subsequent extraction with redistilled petroleum ether. Mice were painted thrice weekly with a 0.5% solution in ether. Certain groups of mice were given small doses of x-rays before injection. The results obtained after 8 months of painting indicate that some mice receiving extract from cancerous Bantus developed skin tumors (12 of 63). Only 3 of these tumors were malignant at the time of writing. Of 41 mice receiving extracts from noncancerous Bantus, 2 developed tumors. One of these was malignant. The control mice receiving extracts from noncancerous Europeans were negative. Mice in the parallel irradiated groups showed a low incidence of induced skin tumors, comparable to the nonirradiated groups.—L. L. W.

EVERETT, J. L., and C. L. HEWETT. (The Chester Beatty Research Inst., The Roy. Cancer Hosp. (Free), London, England.) Polycyclic aromatic hydrocarbons. Part XXV. 1- and 2-alkyl derivatives of 3:4-benzphenanthrene. J. Chem. Soc., 1159-1162. 1940.

In Part XXII (*ibid*, p. 293) the carcinogenic action of several derivatives of 3,4-benzphenanthrene was recorded. The results indicated that substitution in the 1- and 2-positions gave the greatest activity. Hence further derivatives of this type (1-acetyl, 1-ethyl, 1-n-propyl, 1-isopropyl, 2-acetyl, 2-ethyl, 2-n-propyl) have been prepared and are under biological test.—E. L. K.

HEWETT, C. L., and R. H. MARTIN. (The Chester Beatty Research Inst., The Roy. Cancer Hosp. (Free), London. England.) Polycyclic aromatic hydrocarbons. Part XXVI. 1:2:3:4-tetramethylphenanthrene. J. Chem. Soc., 1396-1398. 1940.

After failure by two other methods, this compound, which is related to carcinogenic derivatives of all the 3 compounds 1,2-benzanthracene, 3,4-benzphenanthrene, and chrysene, was synthesized from 1,2,3,4-tetraphenylacetic acid by the Pschorr reaction; biological tests are in progress.—E. L. K.

HIEGER, I. (The Chester Beatty Research Inst., The Roy. Cancer Hosp. (Free), London, England.) The examination of human tissue for carcinogenic factors. Am. J. Cancer, 39: 496-503. 1940.

Variously prepared extracts of livers from Europeans and Bantus with cancer but without primary liver tumors or liver metastases were injected into stock mice. Eleven spindle cell sarcomas developed at the site of injection in 367 mice. Ten of the 11 tumors occurred in females. Extracts of adrenals and prostate, similarly prepared and administered gave negative results. Most of the tumors occurred in mice receiving extracts of the unsaponifiable liver fractions. Feeding of 325 rats and mice on a diet containing Javan *chilies* for 25 months resulted in 1 poorly defined hepatoma, which may have arisen spontaneously.—L. L. W.

HOCH-LIGETI, C. (Roy. Cancer Hosp. (Free), London, England.) Further experiments on the effect of carcinogenic hydrocarbons on the elimination of congo red from the circulation. Am. J. Cancer, 39:365-376. 1940.

In these experiments, continuing the work of T. Csato, C. Wetzler-Ligeti, and B. P. Wiesner (Am. J. Cancer, **36**:257 and 262. 1939) the effects of the prolonged administration of carcinogenic and noncarcinogenic hydrocarbons on the rate of elimination of Congo red from the blood streams of rabbits were compared. The carcinogenic hydrocarbons 1,2,5,6,dibenzanthracene, methylcholanthrene, and 3,4-benzpyrene produced distinct fluctuations in the rate of disappearance of the dye while related noncarcinogenic hydrocarbons did not. It was further found that blood extracts from rabbits treated over a long period with carcinogenic hydrocarbons, when injected into "secondary" test rabbits did not produce a normal acceleration of dye elimination but in some cases retarded it. On the other hand, blood from rabbits treated with noncarcinogenic hydrocarbons produced accelerating effects on their secondary test animals.—L. L. W.

KLEIN, A. J., and W. L. PALMER. (Univ. of Chicago, Chicago, Ill.) Experimental gastric carcinoma. A critical review with comments on the criteria of induced malignancy. Arch. Path., 29:814-844. 1940.

A review of the literature discloses no reliable method of inducing adenocarcinoma of the stomach in animals; in fact, there is no well established case of an adenocarcinoma of the stomach produced experimentally. Some success has attended efforts to produce squamous cell carcinoma in the forestomach in mice and rats. The criteria of induced malignancy are considered. Proof of malignancy is not given by the histologic appearance of a tumor, but by the production of metastases, invasion of neighboring tissues, irreversibility of these activities which must continue in the absence of the extrinsic factor, and the malignant change must occur with sufficient regularity to establish a causal relationship to the experimental procedure.—H. G. W.

KLEINENBERG, H. E., S. A. NEUFACH, and L. M. SHABAD. (Leningrad Branch of the All-Union Inst. for Exper. Med.) Endogenic blastogenic substances. Am. J. Cancer, 39:463-488. 1940.

Ever since the structural similarity of some cancerogenic hydrocarbons and the sex hormones, bile acids, and cholesterol has been known, there has been much speculation as to whether or not the body is able within itself to produce substances capable of bringing about neoplastic changes in its own cells. It is to such, as yet hypothetical, "endogenic blastogenic substances" that the authors refer.

The existence of these substances has been investigated as follows:—Livers from human cancerous and noncancerous patients were obtained at autopsy and benzene extracts of these made. After concentration of the extracts by removal of the benzene, the remaining fatty liquid was injected subcutaneously into white mice of an inbred laboratory strain (R. V.) and into mice of unknown origin from the stock of the Institute for Experimental Medicine. In some of the experiments, extracts that had been distilled at 140°-150° C. for 30 minutes were used, and in others extracts of the nonsaponifiable fractions of the original fatty benzene extract were tested. Olive oil was used as a solvent in still others. Doses of 0.2-0.4 cc. of these extracts were given from 4 to 12 times at intervals of 10, 20, or 30 days, depending on how the substance was tolerated. The injected materials proved very toxic, a large number of mice dying in the first months of the experiment. In the data analyzed, 108 R. V. mice received extracts from the livers of cancer patients, and 54 from the livers of noncancer patients. These animals died after 4 to 6 months on the experiment. The controls consisted of 389 uninjected mice which died at an age of over 8 months.

Of the 108 mice receiving liver extracts from cancerous patients, 24 or 22.2%, developed malignant tumors of some kind. Six of these were at the site of injection (3 sarcomas, 2 adenocarcinomas, 1 carcinosarcoma). Three of the 54 mice receiving extracts from noncancerous patients developed malignant tumors (5.5%). None of these tumors appeared at the injection site. Only 2.3% of the control animals developed spontaneous malignant tumors. Not only was the number of tumors increased in the experimental series, but more varieties appeared than in the controls. Spindle cell sarcomas, 2 types of mammary carcinomas, adenomas of sebaceous glands, squamous carcinomas of the jaws, 2 types of liver tumors and varied forms of lung tumors are described with accompanying microphotographs.

In discussion, the authors attempt to show that the carcinogenic activity of the extracts was due to the endogenic blastogenic substances in the livers of the patients dying of cancer. The malignant tumors arising in mice receiving extracts from noncancerous patients are explained by stating that either all human liver contains "blastogenic substances" or that these patients were "potentially cancerous," and would have developed cancer had they lived, since the endogenic substance was present in their livers.—L. L. W.

LEVINE, M., and H. BERGMANN. (Montefiore Hosp., New York, N. Y.) The cytology of the 1,2,5,6-dibenzanthracene mouse tumors. Am. J. Cancer, 39:504-520. 1940.

A cytological study of 1,2,5,6-dibenzanthracene sarcomas induced in an inbred strain of albino mice was made. One fibrosarcoma in the 8th transplant generation became morphologically a liposarcoma, but gradually reverted to a fibrosarcoma type in succeeding generations. The morphology of the tumors produced is described and photomicrographs are presented. Filtrates of

cultures of *Pseudomonas tumefaciens* were injected into tumor-bearing mice. Hemorrhage and necrosis in the tumors was noted, but no regression.—L. L. W.

LORENZ, E., and H. L. STEWART. (National Cancer Inst., Bethesda, Md.) Intestinal carcinoma and other lesions in mice following oral administration of 1,2,5,6,-dibenzanthracene and 20-methylcholanthrene. J. Nat. Cancer Inst., 1:17-40. 1940.

Ninety-nine mice of strain A and strain A backcross were given aqueous olive oil emulsions containing either 0.2 or 0.4 mgm. 1,2,5,6-dibenzanthracene or 20-methylcholanthrene per cc. instead of drinking water. On this regime, the daily dose per mouse varied from 0.5 mgm. to 0.88 mgm. of hydrocarbon per day. The experiment was continued from 7 to 13 months for some groups of animals. When the mice died or were sacrificed, their organs were studied for possible pathology, and spectrographically for content of carcinogenic hydrocarbon. Dibenzanthracene was found unchanged in the gastrointestinal tract proximal to the ileocecal valve. None was found unchanged distal to this point, and none could be detected in the blood, ascitic fluid, or in any of the body tissues. The most important pathological findings were 10 adenocarcinomas of the small intestine, multiple primary lung tumors in many animals, atrophy of the hematopoietic and genital tissues, and anasarca. Only 1 mouse showed cirrhosis of the liver. The intestinal tumors were first observed after 7 months and a total dosage of dibenzanthracene or methylcholanthrene ranging from 113 to 174 mgm. per mouse. The paper contains tables, charts, and photomicrographs.—L. L. W.

MIDER, G. B. (National Cancer Inst., Bethesda, Md.), and J. J. MORTON (Univ. of Rochester Sch. of Med. and Dentistry, Rochester, N. Y.). Relative importance of local and constitutional effects of methylcholanthrene in production of skin tumors in the mouse. J. Nat. Cancer Inst., 1:41-44. 1940.

Young mice of the C57 black and C57 brown strains which show a low spontaneous incidence of skin tumors, were given biweekly paintings of a 0.5% solution of methylcholanthrene in benzene. Other groups of the same strains were given a single painting of the carcinogen, or were given a single painting on the back and additional paintings thereafter at other sites. A final group of C57 brown mice was painted once with methylcholanthrene and then given one intraperitoneal injection of 2.0 mgm. methylcholanthrene in sesame oil. The number and life histories of the papillomas arising in the various groups were compared. Papillomas produced by a single application of the hydrocarbon to C57 black mice disappeared spontaneously, but repeated applications resulted in papillomas that persisted and in many instances became malignant. Single applications of methylcholanthrene to the backs of C57 brown mice also produced a number of papillomas that regressed but a few became carcinomatous. Additional painting at distant sites, or intraperitoneal injections of the carcinogen did not affect the incidence or behavior of the skin tumors arising at the primary site of application. The mice were studied in groups of 28 to 156 animals each.—L. L. W.

MORI, K., and W. NAKAHARA. (Lab. of the Japanese Foundation for Cancer Research, Tokyo.) Effect of liver feeding on the production of malignant tumors by injections of carcinogenic substances. Gann, 34:48-58. 1940.

Liver feeding definitely inhibited the progress of tissue changes leading to the production of liver cancer induced by repeated intraperitoneal injections of dimethylaminoazobenzol. The inhibiting effect of liver feeding upon carcinogenesis in tissues other than liver was not demonstrable in 2 experiments based on subcutaneous injections into rats of methylcholanthrene and on intraperitoneal injections into mice of 3,4-benzpyrene. These results are in line with those of previous workers on the production of skin cancer in mice by coal tar painting, and indicate that the inhibiting action of liver feeding may be limited to the case of cancer production in the liver.—S. B-J.

NAGAO, N. (Sasaki-Lab., Tokyo.) Über die Fütterungsversuche mit 4'Amino-2:3'-dimethylazoxybenzol bei weissen Ratten. The effect of feeding white rats 4'-amino-2:3'-dimethylazoxybenzol. Gann, 34:13-20. 1940.

This compound administered in olive oil by mouth to white rats had a definite effect upon the liver but did not act as a carcinogen. The introduction of an oxygen atom in the azo-position in o-aminoazotoluol causes a loss of the carcinogenic action of the compound upon the liver and the urinary bladder. Thrombosis and congestion of the liver were produced. The chemical method for preparing this compound is described. The lesions are illustrated in 10 figures.—S. B-J.

SALL, R. D. (Harvard Med. Sch., Boston, Mass.), and M. J. SHEAR (National Cancer Inst., Bethesda, Md.), with the assistance of J. LEITER, and A. PERRAULT. Studies in carcinogenesis. XII. Effect of the basic fraction of creosote oil on the production of tumors in mice by chemical carinogens. J. Nat. Cancer Inst., 1:45-55. 1940.

Tumors were produced in A strain mice by 3,4-benzpyrene, 1,2,5,6-dibenzanthracene, 20-methylcholanthrene, 10-methyl-1,2-benzanthracene, 9,10-dimethyl-1,2-benzanthracene, and 5-$CONH_2$-10-methyl-1,2-benzanthracene. To the solutions of carcinogenic substances, varying percentages of the basic fraction of creosote oil were added except in the control groups. Then by comparing in this test and control animals the number of tumors produced and their times of appearance, the effect of the creosote oil could be determined. Painting with benzene solutions containing 0.05 to 0.02% 3,4-benzpyrene plus 1% basic fraction of creosote oil accelerated tumor development in female strain A mice. Single subcutaneous injections of lard solutions of benzpyrene and the other hydrocarbons plus 2% of basic creosote oil fraction gave a similar accelerating effect in both male and female A mice if the carcinogen was not in too high concentration. Concentrations of hydrocarbons between 0.02 and 0.1% produced tumors slowly in about 50% of the controls and did not obscure the promoting effect of the creosote oil. Higher concentrations (5%) of the creosote substance apparently had a retarding effect.—L. L. W.

SHIMKIN, M. B., and H. B. ANDERVONT. (National Cancer Inst., Bethesda, Md.) Comparative carcinogenicity of three carcinogenic hydrocarbons. J. Nat. Cancer Inst., 1:57-62. 1940.

A uniform experimental procedure for the determination of relative potencies of carcinogenic hydrocarbons is presented. The principles involved are: use of an inbred strain of mice, single injection of a pure substance in an innocuous solvent, the confirmation of the gross diagnosis of tumor by histological examination, and a comparison of the latent times. The method was demonstrated by testing the carcinogenicity of 20-methylcholanthrene, 3,4-benzpyrene, and 1,2,5,6-dibenzanthracene on mice of the C3H strain. From 0.25 to 2.0 mgm. of each of these hydrocarbons was suspended or dissolved in 0.2 cc. of tricaprylin and injected subcutaneously into 20 C3H mice. Similar injections were given stock albino mice. Clear-cut differences in the latent periods of tumor production for the 3 substances were obtained. Methylcholanthrene produced tumors more rapidly than benzpyrene, and benzpyrene more rapidly than dibenzanthracene. The latent times of tumor production in non-homozygous mice and the numbers of tumors occurring were quite irregular and only grossly indicated the relative potencies of the carcinogenic substances. A supplementary experiment with dibenzanthracene showed that divided doses of hydrocarbon were no more efficacious than a single injection in eliciting sarcomas, providing the total dose was the same.—L. L. W.

TAKIZAWA, N. (Path. Lab. des Inst. für Krebsforschung, Tokyo). Experimentelle Erzeugung des Sarkoms bei der Maus durch die Injektion von Glucose, Fructose und Galactose. Ein Beitrag zur Frage der Histogenese des fibroplastischen Sarkoms. Experimental production of sarcoma in the mouse by injection of glucose, fructose, and galactose. Gann, 34:1-5. 1940.

The injection of 0.75 cc. of a 25% aqueous solution of glucose subcutaneously into mice, daily or every other day, for 250 days caused the development of spindle cell sarcoma at the site of injection in 5 out of 18 mice. This tumor was transplantable. Spindle cell sarcoma was produced also by subcutaneous injection of a 25% solution of fructose, in 2 out of 16 mice. In 4 out of 13 mice spindle cell sarcoma was produced by subcutaneous injections of 25% solution of galactose. The gross and microscopic appearance of the tumors are shown in 18 illustrations.—S. B-J.

VASSILIADIS, H. C. (Cancer Inst., Athens.) Carcinogenic properties of ortho-amidoazotoluol. Am. J. Cancer, 39:377-378. 1940.

In an attempt to repeat Yoshida's (Virchows Arch. f. path. Anat. **283**:29. 1932) production of liver tumors in the white rat, 100 rats were given daily doses of 0.01 gm. of ortho-amidoazotoluol in olive oil. The chemical was added to a diet of wheat flour. At the end of a year no liver tumors were found in any of the animals. In a 2nd series of animals a weekly injection of thorotrast was given in addition to the ortho-amidoazotoluol with the idea of irritating the liver. Still no liver tumors were produced. In a 3rd series where the diet consisted solely of rice and dye substance, a large number of adenocarcinomas of the liver appeared, as Yoshida had previously observed.—L. L. W.

Hormones

BONSER, G. M., and J. M. ROBSON. (Dept. of Exper. Path. and Cancer Research, Univ. of Leeds, England, and Dept. of Pharmacol., Edinburgh, Scotland.) The effects of prolonged oestrogen administration upon male mice of various strains: Development of testicular tumours in the Strong A strain. J. Path. & Bact., 51:9-22. 1940.

The paper describes the results of prolonged estrogen administration in male mice of 3 strains (R III, CBA, Strong A). Animals from the first 2 strains received triphenylethylene or estradiol dipropionate, while those from the 3rd received triphenylethylene only. The results were as follows:

Over 60% of R III males treated for 20 weeks or more with either estrogen developed mammary cancer, whereas no mice of the other 2 strains developed this type of cancer.

Hernia appeared in 23 of 30 R III males treated with triphenylethylene, but in only 6 of 31 treated with estradiol dipropionate. Eleven of 23 Strong A males developed scrotal hernia. No hernias were observed in CBA males.

Advanced atrophy, with arrest of spermatogenesis, occurred in all R III and CBA males treated until the time of death. In no R III male was there any interstitial hyperplasia, but in ⅓ of the CBA males the interstitial cells were larger and more abundant than normal. This change was slight as compared with changes in Strong A males.

In Strong A males treated with triphenylethylene and examined before the expiration of 49 weeks of treatment (7 animals) the testes showed no gross diminution in size. In all the testes examined from treated Strong A mice (20 animals) interstitial hyperplasia was observed. Thirteen animals died or were killed between the 50th and 69th weeks of treatment, and all showed testicular nodules (bilateral in 5 cases). Microscopically the process commenced as a focus of densely packed polygonal cells, and in advanced stages appeared to be malignant. There was no conclusive evidence of invasion or metastasis. Attempts to graft the tumors were unsuccessful.

A progressive metaplasia, commencing in the coagulating gland and involving the seminal vesicles, was produced in both R III and CBA mice. In Strong A males, however, there was a striking absence of squamous metaplasia and keratinization in the pelvic organs.

The adrenals of 44 treated R III mice all showed brown degeneration. Of 30 CBA mice, 11 showed no brown degeneration, in 17 the lesion was very slight and in 2 it was fairly marked. Of 19 Strong A males, 6 showed no brown degeneration and in 13 it was very slight.

Gross pituitary enlargement was noted in 5 R III males (none of which bore a mammary cancer). Of 20 other males (15 of which had mammary carcinomas) the pituitary was of normal size in 19. Thirty of 32 CBA males had pituitaries of normal size. No pituitary enlargement was observed in 21 Strong A males treated with triphenylethylene.

The paper includes a full discussion of the significance of the testicular changes in relation to similar conditions in animals and man. The limitation of the development of experimentally induced testicular tumors to 1 strain of mice points to the importance of genetic constitution in determining the origin of these tumors.—A. H.

FERGUSON, R. L., R. D. TEMPLETON, M. C. PATRAS, and F. A. McJUNKIN. (Loyola Univ. Sch. of Med., Chicago, Ill.) Effects of thyroid and calcium therapy on the growth of sarcoma transplants in thyroparathyroidectomized rats. Arch. Path., 29:785-789. 1940.

The influence of thyroid on tumor growth was proportional to its effect on the general metabolism, and the addition of 1% calcium carbonate to the diet had no significant effect on the growth of the host or of the sarcoma transplant.—H. G. W.

ITO, S. (Lab. des Gunsei-Krankenhauses.) Studien über Geschwustentstehung und innere Sekretion. Mitteilung II. Hepatomentstehung und Prolan. Relation of prolan to production of hepatoma. Gann, 34:31-32. 1940.

The author noticed previously that removal of the ovaries of rats developing hepatoma produced by o-amidoazotoluol restrained the malignant changes in the tumor. In experiments in which the animals were fed o-amidoazotoluol in olive oil for 365 days some groups of female rats were castrated; others were injected with prolan. Prolan definitely hindered the development of hepatoma.—S. B-J.

LEWIS, A. A., and C. W. TURNER. (Dept. of Dairy Husbandry, Univ. of Missouri, Columbia, Mo.) The effect of estrogens and a carcinogenic chemical in stimulating the secretion of the mammogenic duct growth factor of the anterior pituitary. Cancer Research, 1:55-59. 1941.

Of various estrogens promoting mammary gland proliferation, the most active, as judged by the mammogenic mouse unit technic, was found to be stilbestrol, which proved to be at least 400 times as effective as estrone. Next in activity, among the compounds studied, was estradiol benzoate, which showed 240 times the effectiveness of estrone, by weight. Anol and triphenyl ethylene compared favorably with aqueous estriol, but were only 1/30 as effective as estrone. 1,2,5,6-Dibenzanthracene was only 1/75 as effective as estrone.—Author's summary.

MARINE, D., and S. H. ROSEN. (Montefiore Hosp., New York, N. Y.) Increase in the incidence of lymphomatosis in male fowls by castration. Am. J. Cancer, 39:315-318. 1940.

Lymphomatosis characterized by widespread tumor infiltrations in viscera and skin occurred in 16 of 119 caponized S. C. white Leghorn cockerels. In none of the birds developing the disease could active testicular fragments be found at autopsy. On the other hand, in 55 birds in which the castrating operation was incomplete, no clear-cut case of lymphomatosis was encountered. A relationship between the development of the lymphomatous condition and a lack of male hormones was thus suggested.—L. L. W.

SALTER, W. T., I. R. NATHANSON, and H. WILSON. (Collis P. Huntington Memorial Hosp., Thorndike Memorial Lab., Boston City Hosp., and the Dept. of Med., Harvard Med. Sch., Boston, Mass.) Experimentally induced benignancy of neoplasm. V. The influence of hormones on the host's resistance to implanted neoplasm. Cancer Research, 1:60-64. 1941.

In a previous report (Arch. Path., **27**:828-840. 1939) it was shown that injections of estrogen changes "the fitness of the animal host as an animated culture medium for supporting the continued growth of a small island of tumor consisting of cells already clearly malignant." In the present experiments sarcoma 180 was implanted in mice of the C57 black and Bagg albino strains. Groups of the tumor-bearing animals were then injected with estrone, thyroxine, testosterone, and anterior pituitary growth hormone. The effects of castration also were studied. Under certain conditions estrone inhibited the growth of the implanted sarcoma. In this case, the sarcoma behaved as if benign in several respects. The inhibition is probably not a direct inhibition of tumor tissue, but the enhancement of a primary resistance mechanism. Androgen, anterior pituitary growth hormone, and thyroxine did not have this inhibitory effect. Castration of females may decrease slightly the resistance to tumor growth.—S. B-J.

SCHÖNBERG, A., J. M. ROBSON, W. TADROS, and H. A. FAHIM. (Fouad the 1st Univ., Cairo, Egypt, and Dept. of Pharmacol., Univ. of Edinburgh, Edinburgh, Scotland.) Synthetic estrogens related to triphenylethylene. J. Chem. Soc., 1327. 1940.

In view of the remarkable estrogenic activity of triphenylchloroethylene, which has been applied clinically, a number of new compounds related to triphenylethylene were synthesized, of which one was much more active than triphenylchloroethylene, as is shown in the following table:

	Dose mu g.	Duration in days till intensity of estrus falls to ½	Proportion of mice showing full vaginal cornification
αα-Di-p-methoxyphenyl-β-phenylbromoethylene	100	68	3/3
	20	9	10/10
	5	Slight effect	0/5
Triphenylchloroethylene	100	12	11/15
	50	No effect	0/10
Stilboestrol	500	16	10/10
	50	9	10/10

The triphenylethylene compounds have a prolonged action, although the threshold dose is comparatively high.—E. L. K.

SHIMKIN, M. B., and H. G. GRADY. (National Cancer Inst., Bethesda, Md.) Carcinogenic potency of stilbestrol and estrone in strain C3H mice. J. Nat. Cancer Inst., 1:119-128. 1940.

Virgin female and male C3H mice were divided into groups of 5 or 10 animals, so that although the sexes were separate, litter mates were present in each group. The mice were then given weekly subcutaneous injections of stilbestrol in sesame oil and estrone in peanut oil over a period of 24 weeks. The total dosage of stilbestrol in 2 groups of males and females was 5 mgm. and 1.2 mgm. respectively. One group of males and females received 1.2 mgm. of estrone and 10 females were left untreated. The average latent period of the tumor development in the controls (untreated females) was 46.5 weeks, in the stilbestrol treated animals 34.8 weeks and in the estrone injected animals, 30.3 weeks. Eight of 10 male mice receiving a total dose of 5.0 mgm. of stilbestrol developed mammary carcinoma as definite evidence of the carcinogenicity of this substance. Histological observations on the tumors produced, the endocrine organs, and the genital tissues are presented.—L. L. W.

TANAKA, A., and S. TUBOI. (Path. Inst. d. med. Akademie, Kyoto.) Über den Einfluss des β-Indolylessigsauren Kaliums auf das Wachstum des Mäusecarcinoms. The effect of potassium β-indolacetate on the growth of carcinoma in mice. Gann, 34:354-355. 1940.

Investigations were made of the influence of potassium salts of the phytohormone (heteroauxin), potassium β-indolacetate on the growth of Bashford's carcinoma. The lethal dose of this compound per 10 gm. of body weight for mice weighing 15 gm. was 15 mgm. by subcutaneous injection and 10 mgm. by intravenous injection. Three groups of mice were injected subcutaneously respectively with 0.03 mgm., 0.3 mgm., and 3.0 mgm. of potassium β-indolacetate 10 days after subcutaneous implantation of the carcinoma. The tumors grew in all animals but were smaller in the animals injected with this compound than in the controls. It is concluded that potassium β-indolacetate hinders the growth of this transplanted carcinoma in mice. Reference is made to similar inhibitory effects of indole, skatole, and tryptophane.—S. B-J.

Von Haam, E., and L. Cappel. (Ohio State Univ., Columbus, Ohio.) Effect of hormones upon cells grown in vitro. I. The effect of sex hormones upon fibroblasts. Am. J. Cancer, 39: 350-353. 1940.

The effect of low concentrations of estrin, progesterone, and testosterone on the growth of mouse embryo fibroblasts in tissue cultures was studied. Estrin in a dilution of 10^{-7} had a slight stimulatory effect, but higher concentrations were inhibitory as were all concentrations of progesterone and testosterone.—L. L. W.

Von Haam, E., and L. Cappel. (Ohio State Univ., Columbus, Ohio.) Effect of hormones upon cells grown in vitro. II. The effect of the hormones from the thyroid, pancreas and adrenal gland. Am. J. Cancer, 39:354-359. 1940.

Thyroxin in concentrations of 10^{-7} and 10^{-9} increased slightly the growth of fibroblasts from mouse embryo hearts as compared to the Tyrode controls. Insulin added to flask cultures produced a 1000% increase of the original explant within 7 days with a large rise in the mitotic index. Concentrations of 0.001 unit and 1 unit per cc. were used. With insulin it was noted that lipoid droplets accumulated in the fibroblasts, making them nearly indistinguishable morphologically from fat cells. Adrenalin and cortin in all dilutions used proved inhibitory to the growth of fibroblasts. Cortin, however, was found to prolong the life of the culture.—L. L. W.

Viruses

DURAN-REYNALS, F. (Yale Univ. Sch. of Med., New Haven, Conn.) Neutralization of tumor viruses by the blood of normal fowls of different ages. Yale J. Biol. & Med., 13: 61-76. 1940.

Humoral antibodies against the Rous chicken sarcoma develop with maturation. The serums of chickens 6-10 months old reduced the activity of tumor extracts, diluted from 1:20 to 1:200, when incubated together for 3 hours at 2-4° C. The incidence of takes was lower and the size of the tumors smaller at 21 days in comparison with control extracts similarly incubated with saline. The tumor growth rate was greater in chicks than in mature animals. Mature animals exhibited marked variations in susceptibility. The serums of 2 resistant chickens had a greater inhibitory effect than 1 from a known susceptible. That the action of the inhibiting serums was directly on the virus was demonstrated by the use of virus material purified by ultracentrifugation. Furthermore the capacity of the serums to flocculate extracts of the tumor as well as normal mouse liver bore no relation to inhibiting potency. The serums of adult chickens also inhibited the virus of the Fuginami sarcoma and that of Mill Hill 2 endothelioma.—A. A. L.

DURAN-REYNALS, F. (Yale Univ. Sch. of Med., New Haven, Conn.) A hemorrhagic disease occurring in chicks inoculated with the Rous and Fuginami viruses (with a section on histopathological findings by Robert M. Thomas). Yale J. Biol. & Med., 13:77-98. 1940.

Among the determinants of the tissue response to the Rous and Fuginami agents are the host's age and the dose and activity of the virus. Intrajugular injection of tumor extracts diluted from 1:20 to 1:2000 in amounts proportional to the body weight, produced generalized hemorrhagic, neoplastic, or mixed lesions. The 1st, which was rapidly fatal, predominated in the youngest chicks (in whom no viral antibody was present), the 3rd was characteristic of mature animals, and the 2nd occurred in those of intermediate age. The hemorrhagic changes, most often observed in the liver and spleen, sometimes occurred in the absence of demonstrable tumor tissue and were interpreted as resulting probably from damage to the endothelium directly. It was concluded that the hemorrhagic disease was not due to an adventitious virus, since after 8 passages through chicks typical neoplastic lesions were still elicited in adults. When subcutaneous lesions developed at the site of intravenous inoculation they were most often of the same type as the generalized. In the more susceptible animals of all ages in which the tumor grew rapidly, the lungs and other metastatic sites tended to contain hemorrhagic tumors.—A. A. L.

DURAN-REYNALS, F. (Yale Univ. Sch. of Med., New Haven, Conn.) Production of degenerative inflammatory or neoplastic effects in the newborn rabbit by the Shope fibroma virus. Yale J. Biol. & Med., 13:99-110. 1940.

Intracardiac injection of fresh saline extracts of the Shope fibroma produced in newborn rabbits a generalized disease characterized by vacuolization, and rarely necrosis, chiefly of the cells of the kidney and liver. Sometimes these were associated with discrete hemorrhages and cell proliferation, particularly in the spleen. Virus was demonstrated in the viscera. Adult animals injected intravenously with proportionate doses developed no noticeable disease. Subcutaneous inoculation of newborn rabbits produced large invasive tumors which exhibited a tendency to form satellite nodules, together with generalized degenerative changes as after intracardiac injection. Virus previously stored in glycerine induced similar changes in some newborn rabbits. Others developed multiple visceral as well as local tumors, in one instance together with degenerative renal change. Some rabbits showed local tumors which regressed, or no tumors or visceral lesions. In contrast, in adult rabbits, subcutaneously introduced fresh virus elicited merely local fibromas, and glycerinated virus none at all.—A. A. L.

Genetics

HESTON, W. E. (National Cancer Inst., Bethesda, Md.) Lung tumors and heredity, the susceptibility of four inbred strains of mice and their hybrids to pulmonary tumors induced by subcutaneous injection. J. Nat. Cancer Inst., 1:105-111. 1940.

Strain A mice which are susceptible to induced lung tumors were outcrossed to strains L, N, and W which are relatively resistant. Tumors were induced in the parent stocks, in their progeny, and in the progeny of the backcrosses by the subcutaneous injection of dibenzanthracene and the incidence studied. The data obtained do not support the theory that the action of a single dominant Mendelian factor governs the susceptibility to induced lung tumors in mice. They suggest rather the action of multiple factors, some probably nongenetic in origin. It was further shown that there is no linkage between susceptibility to lung tumor and the genes for leaden, piebald, dilution, agouti, or brown.—L. L. W.

STRONG, L. C. (Yale Univ. Sch. of Med., New Haven, Conn.) A genetic analysis of the induction of tumors by methylcholanthrene with a note on the origin of the NH strain of mice. Am. J. Cancer, 39:347-349. 1940.

In mice of the NH strain injected subcutaneously with 1.0 mgm. methylcholanthrene in 0.1 cc. sesame oil, various types of tumors or no tumors developed. Offspring of these mice were then selectively bred according to the type of tumor they had produced. In the F3 and F4 generations, litter mates began to develop similar types of tumors, that is, began to show the effects of the selective breeding. Thus a selection line for spindle cell sarcoma, carcinoma of the skin, and for no tumor became well established. Two other selection lines have not yet become pure. The author concludes that the directional path taken by the individual in specific tumor induction is determined by genetic factors.—L. L. W.

Physical Factors

LUCKÉ, B. (Sch. of Med., Univ. of Pennsylvania, Philadelphia, Penn.) Physical factors influencing the growth of cancer. (Experimental studies based on renal adenocarcinoma in animals.) J. Urol., 44:545-558. 1940.

The mechanics of growth of adenocarcinoma of the frog kidney have been studied by transplanting it into the anterior chamber of the frog eye. The form of the growing tumor depends on the immediate physical environment, the growth taking place according to well defined structural patterns. Temperature affects the rate and character of cancerous growth in much the same general way as it affects the growth of normal tissues. Metastasis occurs more often and is more extensive at high temperature than at low.—H. G. W.

Radiation

DALE, W. M. The effect of x-rays on enzymes. Biochem. J., 34:1367-1373. 1940.

Crystalline carboxypeptidase and partly purified polyphenoloxidase are inactivated by x-rays. The rate of inactivation varies with the enzyme concentration. Inactivation does not occur if carboxypeptidase is acting on a substrate during irradiation.—E. B.

FAILLA, G. (Memorial Hosp., New York, N. Y.) Some aspects of the biological action of ionizing radiations. Am. J. Roentgenol., 44:649-664. 1940.

A lecture discussing the effect of radiation on mouse sarcoma, and the enhancing of this effect by subsequent injections of distilled water.—H. G. W.

HIRSHFELD, J. W., R. TENNANT, and A. W. OUGHTERSON. (Yale Univ. Sch. of Med., New Haven, Conn.) The effect of colchicine and x-ray on a transplantable mammary carcinoma in mice. Yale J. Biol. & Med., 13:51-59. 1940.

Consistent effects of colchicine upon the Yale carcinoma 1 were obtained by basing the dose of colchicine upon the animal's weight. Single injections of from 0.009 to 0.0012 mgm./gm. produced a progressive accumulation of cells in an atypical metaphase characterized by contraction of the chromosomes into a compact central mass; this was maximal at about 9 hours. With doses greater than 0.0014 mgm./gm. necrosis was observed in the central portions of the tumor. Single injections as high as 0.0035 mgm./gm. were toxic but not always fatal. When from 0.0035 to 0.0010 mgm./gm. of colchicine were administered repeatedly at intervals of from 3 to 5 days most of the animals died and none of the tumors regressed. With the smaller dose, however, in some instances retardation of tumor growth and prolongation of life were observed. The percentage of cures was higher than with x-ray alone when the 2500 r dose of x-rays was combined with the action of colchicine 0.0016 to 0.0010 mgm./gm. administered 8 to 10 hours before.

But when the dose of x-ray under similar conditions was 5000 r colchicine did not strikingly increase the 48% cures obtained with irradiation alone.—A. A. L.

HOCH-LIGETI, C. (The Chester Beatty Research Inst., The Roy. Cancer Hosp. (Free), London, England.) The effect of prolonged x-radiation on the Congo red index of rabbits. Cancer Research, 1:28-33. 1941.

Treatment with x-rays continued for 1 year induced fluctuations in the Congo red index of the rabbit which range between those of normal rabbits and those seen in rabbits treated with carcinogenic hydrocarbons. Five out of 6 rabbits showed changes in the circulating removal factor resembling those seen in animals treated with carcinogenic hydrocarbons, while in 1 rabbit the removal factor retained the normal positive character.—Author's summary.

HSU, CHIEN-LIANG, and W. C. MA. (Peiping Union Med. Coll., Peiping, China.) Direct and indirect effects of roentgen radiation on the blood-forming organs of rats. Am. J. Cancer, 39:319-333. 1940.

Following a brief review of the literature dealing with the effects of x-rays on hemopoietic tissues, the authors report the microscopic findings in the bone marrow, spleen, and lymph nodes of rats receiving doses of low voltage roentgen radiation over 1 limb. The changes in the marrow of the irradiated limb were of hypoplastic or aplastic nature, whereas the nonirradiated bone marrow and lymph nodes showed hyperplastic responses. Doses of 1,000, 2,500 and 5,000 r delivered in 8, 20, and 40 days respectively were used. Detailed histological descriptions and numerous microphotographs are presented.—L. L. W.

MORTON, J. J., E. M. LUCE-CLAUSEN, and E. B. MAHONEY. Univ. of Rochester, Rochester, N. Y.) The effect of visible light on the development of tumors induced by benzpyrene in the skin of mice. Am. J. Roentgenol., 43:896-898. 1940.

A retardation in the carcinogenic process and a diminution in the number of animals which developed tumors was found in a group of animals exposed to light as contrasted with a group kept in complete darkness.—H. G. W.

WHITMORE, E. R. (Georgetown Univ. Med. Sch., Washington, D. C.) The biologic action of radiant energy. South. M. J., 33:572-580. 1940.

By using carefully measured doses of radiation from a quartz mercury arc lamp, papillomas and squamous cell cancer of the skin have been produced in white mice with metastasis to the lung in 1 case. The effect of sensitization of the skin by *Hypericum* (St.-John's-wort) and buckwheat combined with exposure to sunlight or to quartz mercury radiation are being investigated.—H. G. W.

YAMASHITA, H., K. MORI, and M. MIWA. (Radiolog. and Path. Div. of the Japanese Foundation for Cancer Research, Tokyo.) The action of ionizing rays on sea-urchin. V. The mitotic observations on the effects of roentgen rays upon the unfertilized eggs and sperm. Gann, 34:239-245. 1940.

Two species of sea urchin, *Pseudocentrotus depressus* and *Strongylocentrotus purcherrimus* were used. Irradiation was made at 3 ma. and 170 kv., through a filter of thin aluminum. A batch of unfertilized eggs or sperm was placed at the distance of 17 cm. from the focus of the x-ray tube. The exposure time was 90 minutes. The dose was approximately 12,000 r. Irradiation delayed cleavage of the eggs. The delay in the time of the first cleavage occurred essentially in the nuclear streak phase and prophase in the mitotic cycle. The authors suggest that the nuclear streak phase in the mitotic cycle is involved in the damaging of living cells by irradiation.—S. B-J.

Chemosurgery

MOHS, F. E., and M. F. GUYER. (Dept. of Surg., Wisconsin Gen. Hosp., and Dept. of Zool., Univ. of Wisconsin, Madison, Wis.) Pre-excisional fixation of tissues in the treatment of cancer in rats. Cancer Research, 1:49-51. 1941.

This paper describes a method for using a paste containing zinc chloride for the fixation of a neoplasm *in situ*, under microscopic control. The formula of this preparation (Z-108a) is:

Stibnite (80 mesh sieve)	40.0 gm.
Sanguinaria canadensis	10.0 gm.
Zinc chloride, saturated (4:1) soln	34.5 cc.

In rats, growths of transplanted Philadelphia 1 sarcoma, mammary carcinoma MS-2, Flexner-Jobling carcinoma, and carcinoma C-256 were removed by applications of this paste, followed by excision of fixed tumor tissue. Treatment of cancer in rats by fixative chemicals did not increase metastasis even when this was purposely attempted by prolonged subcurative dosage.—S.B-J.

Biochemistry and Nutrition—Chemotherapy

ANDO, T. (Path. Inst. der Kais. Univ. zu Kyoto.) Einfluss der Futtermittel auf die experimentelle Leberkarzinomentstehung. I. Mitteilung. Experimentelle Leberkarzinomentstehung mit Getreide (Zeitdauer d. Fütterung: 280 Tage). The influence of feeding cereal during 280 days on the experimental production of carcinoma of the liver. Gann, 34:371-373. 1940.

Groups of rats were fed the same amounts of o-amidoazotoluol in the following different diets: polished rice, unpolished rice, rice and yeast, wheat, and barley. The animals in the barley group died in 231 days without cancer of the liver. All 6 animals in the polished rice group developed carcinoma of the liver; 5 had hepatoma and 1 had cholangioma. Of 6 rats on the rice-yeast diet 3 developed hepatoma. None of the 6 animals on the wheat diet developed carcinoma of the liver. The results show the important influence of diet upon the production of carcinoma of the liver by o-amidoazotoluol. Certain cereals, particularly wheat, inhibit the development of tumors after feeding this compound. This coincides with knowledge of the incidence of cancer of the liver in man, the disease being more prevalent among people who have a rice diet than among those living on a wheat diet.—S. B-J.

BOYLAND, E. (Chester Beatty Research Inst., The Roy. Cancer Hosp. (Free), London, England.) Experiments on the chemotherapy of cancer. 4. Further experiments with aldehydes and their derivatives. Biochem. J., 34:1196-1201. 1940.

Compounds were administered by mouth daily to dilute brown mice grafted with the homologous sarcoma M. C. D. B. 1 and to mice bearing spontaneous mammary carcinomas. The growth of the sarcomas was compared with that of control tumors grafted at the same time. The growth under treatment of the carcinomas was compared with the growth of the same tumor during a period immediately preceding treatment. The results have been examined statistically. Heptaldehyde (Strong. Am. J. Cancer, **35**:401. 1939), phloroglucinaldehyde (Boyland and Mawson. Biochem. J., **32**:1982. 1938), and acrolein were all equally effective in checking carcinoma growth. It is possible that heptaldehyde is metabolized by way of pimelic acid, glutaric acid, and malonic acid. Malonic acid markedly inhibited the growth of sarcomas and carcinomas. Citral was much more effective than other aldehydes tested but the known metabolism product "Hildebrandt Acid" was not so active.—E. B.

CARRUTHERS, C. (Barnard Free Skin and Cancer Hosp., St. Louis, Mo.) Influence of heptaldehyde on carcinogenic action of methylcholanthrene. Arch. Path., 30:1184-1191. 1940.

Heptaldehyde markedly inhibits the carcinogenic action of methylcholanthrene when the skin of the backs of mice is

painted with it on alternate days with methylcholanthrene, but has no effect on papillomas and carcinomas that have started to grow.—H. G. W.

CHALKLEY, H. W., and C. VOEGTLIN. (National Cancer Inst., Bethesda, Md.) The effect of variation in oxygen tension and sulfhydryl concentration on nuclear growth and fission in Amoeba proteus. J. Nat. Cancer Inst., 1:63-75. 1940.

Studying *Amoeba proteus* it was found that both nuclear growth and fission can be retarded by either increasing or decreasing the O_2 tension for a period of 24 hours. The retarding effect of an increased O_2 tension was unchanged by the addition of M/100,000 SH glutathione to the medium, but the retarding effect of a decreased O_2 tension was enhanced by its addition. Recovery of nuclear growth and fission took place when the O_2 tension was returned from low to normal but not from high to normal. Amoebae studied in cultures containing SH glutathione at 1/100,000 at normal oxygen tension showed acceleration of both nuclear growth and fission rate. These phenomena were accentuated when O_2 tension was increased for a short period (3 hours) and then returned to normal. The authors suggest that the SH group and oxygen may act upon intracellular enzymes concerned with nuclear growth, which in turn is correlated with fission.—L. L. W.

CRABTREE, H. G. (Imperial Cancer Research Fund, Mill Hill, London, England.) Retardation of the rate of tumour induction by substances which inhibit glycolysis. J. Path. & Bact., 51:303-309. 1940.

A series of mono-chlor-compounds reacted with cysteine with increasing velocity in the following order: monochloracetal, α-chlorhydrin, α-chlordiethylketone, methyl-α-chlorpropylketone, monochloracetone. The ability to inhibit the anaerobic glycolysis of tumor tissue *in vitro* also increased in the same order. Mixed stock mice were painted with carcinogenic compounds (either 3,4-benzpyrene dissolved in ether and liquid paraffin or 1,2,5,6-dibenzanthracene dissolved in acetone and liquid paraffin) twice weekly and with a chlor-compound (0.3% in ether) thrice weekly. The chlor-compounds inhibited tumor induction to a degree varying with their chemical reactivity and their power of inhibiting glycolysis.

Berenblum, Kendal, and Orr (Biochem. J., **30**:709. 1936) had shown that ββ′-dichlorodiethylsulphide, ββ′-dichlorodiethylsulphone and cantharidin inhibit tumor glycolysis and the induction of tar tumors while ββ′-dichlorodiethylsulphoxide, thiodiglycol and croton oil did not reduce glycolysis or the formation of tar tumors.—E. B.

CRABTREE, H. G. (Imperial Cancer Research Fund, Mill Hill, London, England.) Stimulation of tumor induction by an inhibitor of cell glycolysis. Cancer Research, 1:34-38. 1941.

Benzpyrene and mono-chloracetone were applied to the skin of mice in various amounts, and at various time intervals. The effect of mono-chloracetone on the rate of tumor induction by 3,4-benzpyrene in mice is a function of the concentration used. Low concentrations caused marked inhibition, but higher concentrations appear to be innocuous, since the net effect on the average induction time is negligible. Higher concentrations may prove to be slightly stimulatory in mice more susceptible to tumor development. The discussion includes a concept of stimulation in which a residual tendency to carcinogenesis is enhanced by the cooperation of a disturbance of cell metabolism. This metabolic disturbance may be complex. The analogy between the checking of glycolysis *in vitro* and the action *in vivo* may not be sufficiently comprehensive.—S. B-J.

CRABTREE, H. G. (Imperial Cancer Research Fund, Mill Hill, London, England.) Retardation of the rate of tumor induction by hydrolyzing chlor-compounds. Cancer Research, 1:39-43. 1941.

The effect of a series of aliphatic, and one aromatic, acid chlorides (stearyl, acetyl, valeryl, palmityl, myristyl, and benzene sulfochlorides) upon the rate of tumor induction in mice by 3,4-benzpyrene was investigated by the use of local cutaneous applications of the compounds in various concentrations and at various intervals of time. All proved inhibitory. The degree of inhibition varied with the molecular size and rate of hydrolysis, but did not run parallel with any single physical or chemical characteristic. The most effective was benzene sulfochloride which hydrolyzes less rapidly than the aliphatic acid chlorides. It is concluded that the acid chlorides impair the activities of cells nonspecifically due to the liberation of HCl, and that the apparent inhibition of glycolysis is merely one expression of this generalized damage.—S. B-J.

ELLIOTT, K. A. C. (Biochemical Research Foundation of the Franklin Inst., Philadelphia, Penn.) Inhibition of the succinoxidase system by extracts of tumour and normal tissues. Biochem. J., 34:1134-1141. 1940.

Suspensions and extracts of Walker 256 carcinoma, Philadelphia 1 sarcoma, spleen, and pancreas, reduced the oxygen uptake of liver suspensions in the presence of succinate. Suspensions of Jensen rat sarcoma had much less effect. Tumor extracts did not inhibit the reduction of methylene blue by succinic dehydrogenase to the same extent. The effect is possibly due to action on the cytochrome part of the exidase system.—E. B.

GREENSTEIN, J. P., with the technical assistance of W. V. JENRETTE. (National Cancer Inst., Bethesda, Md.) Chemical studies on the components of normal and neoplastic tissues. I. Viscosity and streaming birefringence of sodium thymonucleate. J. Nat. Cancer Inst., 1:77-90. 1940.

This study relates the structural viscosity of solutions of sodium thymonucleate to their streaming birefringence (double refraction of flow) as evidenced by parallel decreases in the presence of certain salts. The most effective anion in this respect is iodide and the most effective cation is guanidinium. The magnitude of the viscosity and intensity of birefringence of the thymonucleate solutions depended largely on the method of preparation. Although the addition of salts to thymonucleate solutions apparently destroyed viscosity and double refraction of flow, these properties returned when the salts were removed. The viscosity of rat serum albumin was increased and that of sodium thymonucleate decreased when the same salts were added to each.—L. L. W.

GREENSTEIN, J. P., with the technical assistance of W. V. JENRETTE. (National Cancer Inst., Bethesda, Md.) Chemical studies on the components of normal and neoplastic tissues. II. The nucleoprotein fraction of normal animal liver. J. Nat. Cancer Inst., 1:91-104. 1940.

A method of separation of nucleoproteins from the livers of rabbits, rats, calves, and cows is described. Analyses of the preparations were made for total N, amide N, P, total S, cystine-cysteine, methionine, tyrosine, and tryptophane. No significant differences between species were found in the values obtained for these substances. However, very great species differences were found in the -SH group values as estimated by porphyrindin titration. Nucleic acid was isolated from 1 of the cow liver nucleoprotein preparations and its viscosity and streaming birefringence compared with those of thymonucleic acid. Further viscosity studies on cow liver nucleoprotein showed that this substance behaves like serum albumin in that its viscosity increases in the presence of salts.—L. L. W.

JACOBI, H. P., and C. A. BAUMANN. (Coll. of Agriculture, Univ. of Wisconsin, Madison, Wis.) The effect of fat on tumor formation. Am. J. Cancer, 39:338-342. 1940.

Groups of 20-25 C albino or stock mice were given high fat diets (20-25%) and were painted with carcinogenic hydrocarbons in order to determine the effect of the fat on the rate of tumor production. The fats used were Crisco, lard, coconut oil, wheat-germ oil, and butter fat. The carcinogens were 3,4-benzpyrene, methylcholanthrene, and 1,2,5,6-benzanthracene. Con-

trol groups received basal diets similar to those of the experimental groups except for the omission of the fat. It was found that in the experimental strains, 1 to 1½ months less was required to develop tumors ("wart-like epitheliomata") in 50% of the animals than in the control series. This acceleration of tumor production could be produced in part by applying oils to the skin of mice on the control diets and then exposing them to the carcinogen.—L. L. W.

JOHNSON, J. M. (National Cancer Inst., Bethesda, Md.) Glutamic acid from normal and cancerous tissue. J. Nat. Cancer Inst., 1:113-117. 1940.

The principal amount of glutamic acid in tumor tissue hydrolysates (Jensen rat sarcoma) and in normal tissues (rat livers) was found to be the natural *l*(+) form. Only small amounts of racemic mixtures were found in hydrolysates of the tumor tissue and the normal tissue controls. Pure *l*(+) glutamic acid, furthermore, could be slightly racemized by prolonged heating with hydrochloric acid. These results are at variance with similar analyses made by Kögl and Erxleben (Ztschr. f. physiol. Chem., **258**:57-95. 1939) on various malignant tissues. These authors believe that relatively large amounts (15.6 to 42.7%) of the *d*(−) form of glutamic acid are present in tumor tissue.—L. L. W.

KISHI, S., and W. NAKAHARA. (Lab. of the Japanese Foundation for Cancer Research, Tokyo.) On provitamin D in experimentally produced rat hepatoma. Gann, 34:252-255. 1940.

Cholesterol crystals were isolated from hepatoma nodules produced in rats by feeding butter yellow, dimethylaminoazobenzol, for 150 days, according to the method of Kinosita. The crystals were examined for their chemical properties, and ultraviolet absorption spectra of their solutions were compared with absorption spectra of solutions of cholesterin crystals from organs and tumors. Four of these absorption spectra are shown in illustrations. Hepatomas experimentally produced in rats by feeding with dimethylaminoazobenzol do not contain provitamin D. In this respect they differ from the Iikubo strain of transplantable hepatoma of the rat. The latter tumor contains provitamin D in a concentration of 7%. Noncancerous parts of hepatoma liver and precancerous cirrhotic liver, like normal liver, do not contain provitamin D.—S. B-J.

LEWISOHN, R., R. LEUCHTENBERGER, and D. LASZLOW. (Mt. Sinai Hosp., New York, N. Y.) Spleen extract in the treatment of transplanted and spontaneous malignant tumors in mice. Surg., Gynec. & Obst., 71:274-285. 1940.

Since transplanted sarcoma could be healed by injection of splenic extracts, which produced considerable enlargement of the spleens, the effect of injection of extracts of these enlarged or "healed" spleens was tried. Of 57 animals with transplanted sarcoma, 39 were cured by injections of the healed splenic extract, while of 85 mice with spontaneous carcinoma of the breast 25 were cured, 13 by healed spleen extract, 5 by immune spleen extract, and 7 by concentrated purified spleen extract.—H. G. W.

MORIGAMI, S., and N. KASIWABARA. (Path. Inst. der Osaka Kais. Univ.) Feinere Zytoplasmastruktur der experimentell erzeugten Hepatomzellen. The finer cytoplasmic structure of experimentally produced hepatoma cells. Gann, 34: 247-251. 1940.

The cytoplasmic structure of explained cancer cells from hepatoma, produced experimentally in rats by administration of dimethylaminoazobenzol, was studied by means of the gelatin-silver impregnation method of Fujita and Fukuda. Cells from a tissue culture 48 hours old gave characteristic appearances of cancer cells as distinguished from normal fibroblasts. The fibrillar structures of the fibroblasts were usually thick, long, and few in number. In contrast, the hepatoma cells contained numerous delicate and short fibrils. Cells which had stopped growing lost this fibrillar structure and became granular. These appearances are shown in 4 figures.—S. B-J.

REIMANN, S. P. (Philadelphia, Penn.) Further studies on the role played by certain normally occurring intracellular chemical compounds in growth and development. Am. J. Roentgenol., 44:444-448. 1940.

A lecture on the influence of certain amino acids and nuclear compounds on normal cell growth.—H. G. W.

SUGIURA, K., and C. P. RHOADS. (Memorial Hosp., New York, N. Y.) Experimental liver cancer in rats and its inhibition by rice-bran extract, yeast, and yeast extract. Cancer Research, 1:3-16. 1941.

The authors investigated various factors which influence the production of liver cancer in rats by feeding butter-yellow, p-dimethyl-amino-azobenzene. Albino rats of the Sherman and Wistar strains were more susceptible to the carcinogenic action of butter-yellow than rats of the Evans strain. The induced liver cancers were of several types: trabecular or solid hepatomas, glandular or cholangiomas, and mixed. Cirrhosis with adenomatous hyperplasia of bile ducts preceded these malignant tumors. Daily ingestion of a small slice of carrot did not affect tumor production but prolonged the lives of the animals and influenced the type of tumor. Rice bran extract (ether soluble) exerted a transitory inhibition on the development of liver cancer. Ether extract of yeast also inhibited tumor development, but to a less extent than the ether extract of rice bran. Butter-yellow did not produce liver cancer in rats fed on a diet of unpolished rice containing 15% of brewer's yeast. The paper is a detailed report of experimental tests of the protective action of dietary constituents derived from rice bran and yeast.—S. B-J.

TANNENBAUM, A. (Tumor Research Lab. of Michael Reese Hosp., Chicago, Ill.) Relationship of body weight to cancer incidence. Arch. Path., 30:509-517. 1940.

A study of the incidence of tumors in mice kept on different diets, and study of insurance statistics, supports the conclusion that both animals and men who are overweight are more likely to develop tumors than those who are average or underweight.—H. G. W.

TENNANT, R., and A. A. LIEBOW. (Yale Univ. Sch. of Med., New Haven, Conn.) The actions of colchicine and of ethylcarbylamine on tissue cultures. Yale J. Biol. & Med., 13: 39-49. 1940.

By correlating mathematically the accurately determined expansion rates in Carrel flasks of colonies of cells from a mouse breast carcinoma (Yale carcinoma 1) with the mitotic counts it was found that colchicine, even in concentrations as low as 1:64 million, diminished the rate at which cells entered mitosis. This was true despite the apparently remarkable accumulation of karyokinetic cells. Morphological studies of normal cells from the breast of the newborn mouse showed that following mitosis under the influence of colchicine cells may attain chromosome numbers exceeding the diploid. This results from dispersion of chromosomes longitudinally split during prophase and subsequent reconstitution of the cell without division of the cytoplasm. Despite profound nuclear changes the cells remain alive as demonstrated by successful growth upon transplantation into animals or fresh media. The effect upon mitosis of ethylcarbylamine, known to inhibit the Pasteur phenomenon (Warburg), is similar to that of colchicine but occurs over a narrower and higher range of concentrations.—A. A. L.

UTZINO, S., and T. CHEN. (Med.-chem. Inst. der Med. Fakultät der Kais. Tohoku-Univ., Sendai.) Über die autolytische Kathepsinwirkung des Sarkomgewebes. The autolytic kathepsin activity of sarcoma tissue. Gann, 34:215-229. 1940.

The autolytic kathepsin activity of the Fujinami chicken sarcoma was greatest at pH 4.6. It was weaker in the earlier

stages after inoculation; stronger in the later stages. The addition of cysteine to the tumor emulsion increased the autolytic activity of the sarcoma. Significant autolytic activity was found in a rabbit sarcoma 30 days after inoculation. This approached the activity of the liver. The degree of autolytic activity of organs was in the following order: liver, kidney, spleen, sarcoma, and muscle. Spleen and sarcoma had approximately the same autolytic activity. Cysteine increased the autolytic activity of rabbit sarcoma, but to a less degree than it aided autolysis of the chicken sarcoma. There was no significant difference between the autolytic activity of tissues and organs from normal rabbits and from sarcomatous rabbits.—S. B-J.

Immunology

CHEEVER, F. S., and C. A. JANEWAY. (Dept. of Bact. and Immunol., Harvard Univ. Med. Sch., Boston, Mass.) Immunity induced against the Brown-Pearce carcinoma. Cancer Research, 1:23-27. 1941.

Rabbits were inoculated intratesticularly and intradermally with the Brown-Pearce carcinoma. After regression of tumors tests of immunity were made by reinoculations into the testis. Serological tests were made for the demonstration of humoral antibodies. To investigate the possibility that nonspecific tissue factors might induce a state of resistance to the growth of tumor cells, rabbit embryo skin was injected intradermally into adult rabbits. In confirmation of Besredka's reports, it was observed that over 90% of intradermal Brown-Pearce carcinomas regressed spontaneously, and that rabbits with intracutaneous tumors, or in which these tumors had regressed, were refractory to subsequent implantations of the tumor. No evidence of humoral antibodies was obtained. Pathological and immunological studies did not explain the fundamental nature of the immunity. The possibility of its being a nonspecific tissue reaction is discussed.—S. B-J.

LEWIS, M. R. (Dept. of Embryol., Carnegie Inst. of Washington, Baltimore, Md.) Immunity in relation to 1:2:5:6-dibenzanthracene-induced sarcomata. Bull. Johns Hopkins Hosp., 67:325-344. 1940.

Among 350 tumors induced by dibenzanthracene in mice of pure inbred strains there were found 6 spindle celled sarcomas that grew when transplanted into mice of certain strains, although they regressed later except in animals of the same strain in which the tumor originated. The growth of a graft of any one of the 6 tumors for as long as 4 days in mice of a strain different from that of the tumor's origin brought about in the alien strain hosts an immunity from the growth of the autograft or another graft of the same tumor, but not from the graft of another tumor. Complete removal of the 1st tumor before the implantation of the 2nd showed that this type of tumor immunity was already established by the 5th day and was not dependent upon the continued presence of the 1st tumor nor upon its regression. When the 1st tumor was not removed, it continued to grow for a number of days in those mice which its own growth for 4 days had rendered unfavorable for the growth of another graft of the same tumor. The development of immunity proved to be dependent upon the growth of tumor cells in the host. The absorption of tumor proteins and of other proteins by mice of susceptible strains failed to bring about immunity. The growth of a graft in mice of the strain in which the tumor originated failed to bring about immunity. Mice of each pure inbred strain could be rendered immune from the growth of all tumors that originated in mice of other pure inbred strains, but these mice remained susceptible to the growth of a graft of those tumors that originated in mice of their own strain. Treating tumors with heat, pressure, and chemicals previous to implantation failed to cause regression in mice of their strain of origin.—H. G. W.

MANN, L. S., and W. H. WELKER. (Coll. of Med. of the Univ. of Illinois, Chicago, Ill.) A specific precipitin antiserum for carcinoma protein. Am. J. Cancer, 39:360-364. 1940.

Antiserum was prepared against human carcinoma (gastric, cervical) proteins by injecting rabbits intramuscularly with the tumor tissue adsorbed on aluminum cream according to the principles of Hektoen and Welker (J. Infect. Diseases, **53**:309. 1933). By this method precipitins for the blood serum proteins necessarily included in the tumor tissue disappeared from the rabbits' serums in from 1 to 6 months. Antiserums so prepared gave positive precipitin reactions with autolysates of malignant tissues in high dilutions whereas negative reactions were recorded with normal tissue autolysates and also by normal rabbit serum on malignant tissue. The serum of carcinomatous patients was set up against the pecipitin serums and of 7 patients with clinical gastric cancer, 4 gave positive reactions. Seven of 8 patients with carcinoma of the cervix were also positive. Serum from normal controls or from noncancerous patients with various disorders were negative. The authors conclude that the proteins of carcinoma tissue have specific antigenic qualities and may be detected in the blood stream of patients by the appropriately prepared antiserum.—L. L. W.

Leukemia

BURCHENAL, J. H. (The New York Hosp. and Cornell Univ. Med. Coll., New York, N. Y.) Experimental studies on the relation of pregnancy to leukemia. Am. J. Cancer, 39: 309-314. 1940.

The offspring of 18 pregnant mice with transmissible leukemia (Akf 5 or Akh 106) failed *in utero,* in the neonatal period, or at the age of 3 months to show leukemia. This finding is in accord with the literature on human leukemia complicated by pregnancy, which reports no authentic case of transmission of the disease from mother to fetus.—L. L. W.

FURTH, J., and W. A. BARNES. (Dept. of Path., Cornell Univ. Med. Coll. and the New York Hosp., New York, N. Y.) Differences between malignant blood cells from induced and spontaneous leukemias of mice. Cancer Research, 1:17-22. 1941.

Leukemia was produced by the administration of carcinogenic chemicals in mice of a leukemic stock (AK), in mice of a stock (Rf), in which spontaneous leukemia is rare, and in hybrids of these stocks. The genetic structure of neoplastic blood cells from induced leukemias was tested in transmission experiments using purebred mice of these stocks. Previous experiments have shown that the spontaneous leukemias arising in these hybrids are, in transmission experiments, similar to the spontaneous leukemias arising in leukemic stock. The present experiments have shown that the induced leukemias differ among themselves. Only 1 of 6 transmissible leukemias induced in hybrids resembled closely those arising spontaneously. Two were nonspecific and could be grafted on both parental leukemic and nonleukemic stock, and 2 could be readily grafted on hybrids but not on either parental stock. It is concluded that cells from spontaneous and induced leukemias may differ genetically. This difference may be explained by assuming that the cells of the induced leukemias are mutants.—S. B-J.

ITO, S. (Lab. des Gunsei-Krankenhauses Ayabe, Kyoto.) Über die Entstehung der Leukämie bei Ratten. The production of leukemia in rats. Gann, 34:305-308. 1940.

Extensive myeloid leukemic metaplasia was found in the liver, kidneys, lymph nodes, spleen, and ovaries of a rat which had been used in experiments made to study the inhibitory effect of methylene blue upon the formation of hepatoma by o-aminoazotoluol. The rats were fed for 250 days a diet of unpolished rice containing 2.5% o-aminoazotoluol in olive oil. After the 250th day 11 rats were living. Six of these were then given unpolished rice, to 100 gm. of which was added 5 gm. of 0.1 to 2.0%

aqueous solution of methylene blue. One of the rats in this group developed myeloid leukemia and died on the 321st day of the experiment. The findings at autopsy with a description of the cells are reported. The author comments on the extreme rarity of spontaneous leukemia in the rat and concludes that the occurrence of the disease in this animal was directly related to the feeding of o-aminoazotoluol followed by feeding with methylene blue. The paper is illustrated with 15 figures.—S. B-J.

KIRSCHBAUM, A., K. G. STERN, and C. W. HOOKER. (Yale Univ. Sch. of Med., New Haven, Conn.) Studies on fowl leukosis. Transfer with fractions obtained by ultracentrifugation of leukemic plasm and bone-marrow extracts. Yale J. Biol. & Med., 13:1-14. 1940.

The agent of fowl leukosis (Furth strain 1) was studied in relation to its properties in the ultracentrifuge, and with regard to host susceptibility. Sedimentation of potent material from diluted heparinized plasma of leukemic animals at 30,000 r.p.m. for ½ to 1 hour was incomplete. At 6,000 r.p.m. no sedimentation was demonstrable. Aggregation of particles in the sediment was indicated, first by the observation that the centrifugate was always less active than the crude extract or plasma (contrary to Claude's experience with the agent of chicken sarcoma), and second by the fact that the sedimented agent was not filtrable through candles that readily allowed the passage of particles in the original leukemic plasma. Animals less than 10 weeks of age were found more susceptible than older birds to leukemic whole blood, plasma, crude marrow extracts, and to ultracentrifugates of the last. With whole blood the breed of the fowl also was found to determine susceptibility. When unfiltered leukemic plasma was inoculated into the amniotic sac at the 7th day, some of the animals developed leukemia after hatching, but inoculations into the albumin were never successful before the 10th day (the time when the bone marrow develops). This suggested either that the hemopoietic elements of the yolk sac can be the site of introduction of the leukemia, or that the virus can survive in certain parts of the embryo for at least 3 days. In animals after hatching the bone marrow always gave the first histological evidence of leukemic change.—A. A. L.

General Reviews

COWDRY, E. V. (Washington Univ., St. Louis, Mo.) The properties of cancer cells. Arch. Path., 30:1245-1274. 1940.

A general review of the differences between cancer cells and normal cells. Chief of these are a loss or lack of polarity, a wide range in variation of nuclear and cytoplasmic structures, a decrease in structural differentiation and dependent specific functional activity, a decrease in dependence on oxygen supply, a decrease in organismal control over cell division and an increase in autonomy of cell division, an increase in transplantability and an increase in invasiveness and ability to outlive cells of invaded tissue.—H. G. W.

VOEGTLIN, C. (National Cancer Inst., Bethesda, Md.) The approaches to cancer research. J. Nat. Cancer Inst., 1: 11-15. 1940.

The author discusses fundamental problems of malignant neoplastic growth and outlines some of the approaches of cancer research.—L. L. W.

COMPARATIVE ONCOLOGY

ENGLE, E. T., and A. P. STOUT. (Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.) Spontaneous primary carcinoma of the prostate in a monkey (Macaca mulatta). Am. J. Cancer, 39:334-337. 1940.

A spontaneously occurring carcinoma of the prostate without metastasis is reported in a very old rhesus monkey.—L. L. W.

MIYADI, T. (Path. Inst. der Osaka Kais. Univ., Osaka.) Über einen Fall von grosser Nierengeschwulst beim Kaninchen. A case of a large tumor of the kidney of the rabbit. Gann, 34:374-375. 1940.

This is a description of an embryonal adenocarcinoma of the kidney of a rabbit. There were no metastases.—S. B-J.

PENTIMALLI, F. (Inst. of Gen. Path., Roy. Univ., Naples, Italy.) Transplantable lymphosarcoma of the chicken. Cancer Research, 1:69-70. 1941.

This is a description of a transplantable, nonfilterable lymphosarcoma which arose spontaneously in a Leghorn chicken.—S. B-J.

WARREN, S., and O. GATES. (Path. Lab. of Collis P. Huntington Memorial Hosp., New England Deaconess Hosp., and Dept. of Path., Harvard Med. Sch., Boston, Mass.) Spontaneous and induced tumors of the guinea pig. Cancer Research, 1:65-68. 1941.

The published accounts of spontaneous and induced tumors in guinea pigs are reviewed. The authors report their observations on 1 spontaneous tumor in guinea pigs and on 2 liposarcomas and 2 fibrosarcomas induced in guinea pigs by subcutaneous injection of 3,4-benzpyrene in glycerine. The guinea pig appears to be resistant to the development of spontaneous tumors, to transplantation of spontaneous and induced tumors, and to the production of induced tumors other than of the gall bladder. Carcinogenic hydrocarbons are less potent in guinea pigs than in mice.—S. B-J.

CLINICAL AND PATHOLOGICAL REPORTS

Etiology

QUIGLEY, D. T. (Omaha, Neb.) Etiological factors in some forms of cancer. Am. J. Roentgenol., 44:110-112. 1940.

General considerations, with particular reference to food deficiencies.—H. G. W.

Diagnosis—General

BRUNSCHWIG, A., R. L. SCHMITZ, and T. H. CLARKE. (Univ. of Chicago, Chicago, Ill.) Intravital staining of malignant neoplasms in man by Evans blue. Arch. Path., 30:902-907. 1940.

Of 30 patients injected with Evans blue, 20 showed selective concentration of the dye in and about the neoplastic foci, with no correlation between the histologic type and ability to localize the dye, which is deposited in the fibroblasts and macrophages and not in the tumor cells themselves. Benign neoplasms did not localize the dye.—H. G. W.

ELY, J. O. (Franklin Inst., Philadelphia, Penn.) A fraction of human urine causing fetus resorption in rats and mice. Am. J. Cancer, 39:379-380. 1940.

Fractionated urine solutions from patients with far advanced cancer were found to cause loss of weight, stupor, vaginal bleeding, and resorption of the embryos when injected intravenously into pregnant rats and mice. Similar but less pronounced results were produced by the urine of a healthy subject. The method of preparation of the urine fraction is given.—L. L. W.

KAPLAN, I. I. (Bellevue Hosp., New York, N. Y.) Early diagnosis of cancer. Am. J. Surg., 50:781-786. 1940.

General review.—H. G. W.

NAKAMURA, H., J. TANAKA, and Y. YOHDA. (Path. Dept. Med. Div., Hokkaido Imperial Univ.) Urinary diagnosis of cancer by using albino-salamander (Report I). Gann, 34: 6-11. 1940.

The melanophore pituitary reaction, using the so-called "albino-salamander," whose hypophysis is cauterized, was applied to various cases of cancer, other tumors, pregnancy, diseases of internal secretion, and inflammation. The test was made by injecting 0.2 to 0.3 cc. of morning urine from a patient sub-

cutaneously into the over-wintered albino larva. A positive reaction is indicated by the supervening black coloration of the skin, caused by expansion of melanophores. The reaction was clearly positive in 41 cases of cancers of uterus, stomach, esophagus, rectum, and other sites, and in 3 cases of sarcoma. It was negative in Basedow's disease, gangrene of the lung, abscess of the liver, etc. Urine from pregnant women gave positive reactions. The authors conclude that this is a simple and quick method for diagnosing cancer.—S. B-J.

SANO, M. E., and L. W. SMITH. (Temple Univ. Sch. of Med., Philadelphia, Penn.) Tissue culture as a diagnostic aid in the identification of atypical tumors. Arch. Path., 30:504-508. 1940.

Tissue culture is recommended as an accessory diagnostic aid.—H. G. W.

Radiation—Diagnosis and Therapy

BELL, A. L. L. (Long Island Coll. Hosp., Brooklyn, N. Y.) The effect of radiation on cancer. Am. J. Surg., 50:776-780. 1940.

General review.—H. G. W.

CHARTERIS, A. A. (Nat. Radium Center, Western Infirmary, Glasgow, Scotland.) The radium treatment of rodent ulcer near the eye. Am. J. Roentgenol., 44:737-746. 1940.

Methods of treating rodent ulcers near the eye, with results in a series of 76 cases.—H. G. W.

CUTLER, M. (Chicago Tumor Inst., Chicago, Ill.) Cancer of the larynx. J. A. M. A., 115:1339-1344. 1940.

Clinical study with demonstration that the radiosensitivity of cancer of the larynx cannot be determined on the basis of the microscopic structure alone, but is more reliably shown by the mobility of the lesion.—H. G. W.

FRIED, C. (Inst. de Radium. Sao Francisco de Assis, Sao Paulo, Brazil.) The effect of fractional roentgen irradiation on several groups of inoperable tumors. Am. J. Roentgenol., 44:401-408. 1940.

Clinical study.—H. G. W.

FRIED, J., and J. GOLDBERG. Montefiore Hosp. for Chronic Disease, New York, N. Y.) Post-irradiation changes in the lungs and thorax. Am. J. Roentgenol., 43:877-895. 1940.

A study of 18 cases followed for periods up to 12 years. Patients subjected to heavy radiation in treatment of cancer of the lung may exhibit marked pulmonary fibrosis and evidence of obstruction to the pulmonary circulation.—H. G. W.

GOIN, L. S., E. F. HOFFMAN, and J. J. CRANE. (Los Angeles, Calif.) Treatment of certain types of bladder tumor by contact x-ray therapy (Chaoul technique). J. Urol., 44: 847-849. 1940.

Experience with 17 cases.—H. G. W.

HOWES, W. E., and H. BOLKER. (Brooklyn Cancer Inst., Brooklyn, N. Y.) Preoperative irradiation of the breast. Am. J. Roentgenol., 44:98-107. 1940.

The technic of such preoperative therapy as is now being practiced at the Brooklyn Cancer Institute is outlined, and complications arising from such treatment and their antidotes are discussed.—H. G. W.

HUNT, H. B. (Univ. of Nebraska Hosp., Omaha, Neb.) Treatment of large protruding carcinomas of the skin and lip by irradiation and surgery. Am. J. Roentgenol., 44:254-264. 1940.

Clinical report.—H. G. W.

LEUCUTIA, T. (Harper Hosp., Detroit, Mich.) Late results in supervoltage roentgen therapy. Am. J. Roentgenol., 44: 586-593. 1940.

A statistical study indicating that supervoltage roentgen therapy is of some benefit in certain types of cancer.—H. G. W.

RUBENFELD, S. (Bellevue Hosp., New York, N. Y.) The radiation treatment of giant follicular lymphadenopathy and its polymorphous cell sarcoma derivative (Symmers' disease). Am. J. Roentgenol., 44:875-883. 1940.

Giant follicular lymphadenopathy presents a characteristic histological picture and is rapidly amenable to roentgen therapy, but the polymorphous cell sarcoma derived from it is radioresistant.—H. G. W.

Skin

BROWN, J. B., and L. T. BYARS. (Washington Univ. Sch. of Med., St. Louis, Mo.) Malignant melanomas, with report of 4 and 7 year cures. Surg., Gynec. & Obst., 71:409-415. 1940.

Clinical study.—H. G. W.

FUKUKEI, I., and T. SHINKAWA. (Saito Surgical Clinic, Nagoya Imperial Univ., Faculty of Med.) A case report of cancer of the right leg resulting from burns of the skin. Gann. 34:33-38. 1940.

The patient, a male 68 years old, suffered repeated burns of the leg during many years, caused by his habit of standing by a four-foot square fireplace in his room and by having a charcoal-burning heater placed at his feet when he was in bed, in the winter. The ulcerated lesions were carcinomatous when the patient was first seen. Metastasis had occurred in the inguinal lymph nodes. The tumor of the skin was removed with a Bovie electrical cautery. The case history is illustrated with 6 photographs.—S. B-J.

GOLDMAN, H. J. (Univ. of Penn. Sch. of Med., Philadelphia, Penn.) Multiple benign cystic epithelioma. J. A. M. A., 115: 2253-2257. 1940.

Report of 10 cases in 1 family of 19 members.—H. G. W.

SACHS, W., and P. M. SACHS. (New York Post-Graduate Med. Sch., New York, N. Y.) Turban tumors. Arch. Dermat. & Syph., 42:15-22. 1940.

Turban tumors, that is, large tumors of the scalp, are of varied histologic structure. The first case in a negro is reported.—H. G. W.

TAUSSIG, J., and G. D. WILLIAMS. (Barnard Free Skin and Cancer Hosp., St. Louis, Mo.) Skin color and skin cancer. Arch. Path., 30:721-730. 1940.

The exposed skin of 100 skin cancer patients was on the average definitely redder and darker to the colorimeter than that of 100 controls, but the unexposed skin of the backs showed no such difference. The patients with skin cancer were more often exposed to the action of the external environment than were the controls. Subjects whose skins on unusual exposure tend to darken and redden, if they show skin cancer, more often exhibit the basal or mixed types, while those who do not so react more frequently show the squamous variety.—H. G. W.

TRAENKLE, H. L. (Lab. of Dermatologic Research, Univ. of Pennsylvania, Philadelphia, Penn.) Epithelioma adenoides cysticum, tricho-epithelioma and basal cell cancer. Arch. Derm. & Syph., 42:822-837. 1940.

Most benign cystic epitheliomas are histologically trichoepitheliomas and the more mature forms can more properly be designated hamartoma than epithelioma. Some so-called cylindromas of the scalp are trichoepitheliomatous.—H. G. W.

WARREN, S., and O. GATES. (Harvard Med. Sch., Boston, Mass.) Cancer of the skin in relation to multiple malignant growths. J. A. M. A., 115:1705-1707. 1940.

On the basis of 1,149 cases of cancer of the skin it is concluded that there is no justification in the recommendation of the induction of skin cancer to protect against the development of cancer elsewhere. In fact, with this type of cancer, as with cancer of the other organs, there is a greater number of multiple cancers than would be expected on the basis of chance.—H. G. W.

Nervous System

BAILEY, O. T. (Dept. of Path., Harvard Med. Sch. Boston, Mass.) Histologic sequences in the meningioma with a consideration of the nature of hyperostosis cranii. Arch. Path., 30:42-69. 1940.

The meningioma is a tumor arising from the arachnoid cell, either in the arachnoid villi or on the surface of the arachnoid. Neither the ectodermal theory of the French neuropathologists nor the fibroblastic theory of Mallory in regard to the origin of this tumor is sufficient to explain completely the variations in histologic structure encountered in a series of these neoplasms. The arachnoid cell is differentiated and is specialized as a covering cell. The differentiation persists in meningiomas with the result that the neoplastic cells require a supporting and nutritive stroma. The stroma of meningiomas consists of several elements, the most constant of which are collagenous connective tissue and blood vessels. The fibroglia fibrils seen in meningiomas belong to the fibroblasts of the stroma and not to the neoplastic cells. Elastic tissue also forms part of the stroma of some meningiomas. Hyperostosis cranii is produced by involvement of the skull by the meningioma. It develops when the stroma of the meningioma is derived from the periostium. The great number of histologic variants of the meningioma depends on relationships of the neoplastic cells, with their tendency to cover whatever they touch, to a complex stroma, which continues to form intercellular materials similar to those which the component parts form under normal circumstances.—H. G. W.

CANAVAN, M. M. (Boston, Mass.) Effects of an anterior callosal glioblastoma multiforme on the entire brain. Arch. Path., 30:122-129. 1940.

Report of case in which the tumor mass replaced brain tissue focally, distorted the hemispheres and increased the vascularity forward of the tumor, but did not destroy myelin peripheral to it.—H. G. W.

DYKE, C. G., and L. M. DAVIDOFF. (Neurological Inst., New York, N. Y.) The pneumo-encephalographic appearance of hemangioblastoma of the cerebellum. Am. J. Roentgenol., 44:1-8. 1940.

The cardinal features for the diagnosis of this tumor are described.—H. G. W.

FOOT, N. C. (Cornell Univ. Med. Coll., New York, N. Y.) Meningioma. Arch. Path., 30:198-211. 1940.

Meningioma appears to be a tumor of composite make-up comprising 2 groups of cells: the meningiocytes, derived from the arachnoid villi, and stroma cells coming from the mesenchymal elements of the pia-arachnoid. Usually the former predominate and it is probable that they represent the cell type of the meningioma. Probably these cells are derived from the neural crests, as shown by their dissimilarity to fibroblasts in morphologic aspects and staining characteristics. From the standpoint of their architecture they more closely resemble growths associated with neural than those associated with pure fibrous tissue. The presence of melanin in members of the same series is stressed and a relationship between pigmented meningeal tumors and meningiomas is tentatively advanced.—H. G. W.

FOOT, N. C. (Dept. of Surg. Path. of the New York Hosp. and the Cornell Univ. Med. Coll., New York, N. Y.) Histology of tumors of the peripheral nerves. Arch. Path., 30:772-808. 1940.

A general review, not suitable for abstracting.—H. G. W.

LIBER, A. F., and J. R. LISA. (Lincoln Hosp. and City Hosp., New York, N. Y.) Stromal tumors of the choroid plexus. Am. J. Clin. Path., 10:710-735. 1940.

Review of literature with report of a meningioma, a lipoma, and cases of nodular hyperplasia.—H. G. W.

LOFTIS, E. L. (Dallas, Texas.) Recklinghausen's disease in identical twins. Arch. Derm. & Syph., 42:657. 1940.

Identical twins, 42 years of age, had multiple lesions present in nearly the same distribution since the age of 8 years.—H. G. W.

MASTEN, M. G. (Univ. of Wisconsin Med. Sch., Madison, Wis.) Teratoma of the spinal cord. Arch. Path., 30:755-761. 1940.

A teratomatous cyst attached to the pia of the lower part of the cervical cord is described, being the 14th case reported.—H. G. W.

MOERSCH, F. P., G. J. LOVE, and J. W. KERNOHAN. (Mayo Clinic, Rochester, Minn.) Melanoma of the central nervous system. J. A. M. A., 115:2148-2155. 1940.

Report of 34 cases of secondary melanoma in the central nervous system, emphasizing the frequency of this complication which was present in 10% of the cases of melanoma.—H. G. W.

NAGAHARA, Y. (Path. Inst. der Tokyo Kais. Univ.) Ein seltener Sektionsfall von Recklinghausenscher Krankheit mit Neurofibromatose der Harnblase. A rare case of von Recklinghausen's disease with neurofibromatosis of the bladder. Gann, 34:317-318. 1940.

Report of the findings at necropsy.—S. B-J.

PARSONS, P. B., and L. PLATT. (Duke Univ. Sch. of Med., Durham, N. C.) Calcification in abdominal neuroblastoma. Am. J. Roentgenol., 44:175-177. 1940.

Two cases are reported.—H. G. W.

WELLS, H. G. (Univ. of Chicago, Chicago, Ill.) The occurrence and significance of congenital malignant neoplasms. Arch. Path., 30:535-601. 1940.

Report of 4 cases of congenital neuroblastoma, with review of the literature on congenital neoplasms. About 250 cases were found that were possibly congenital milignant tumors, but only 66 were established beyond possible doubt. Sarcomas constituted nearly one-half of these tumors, but there are nearly as many instances of neuroblastoma, and a vastly larger proportion of the cases of neuroblastoma are congenital. Not a single case of congenital carcinoma can be found convincingly established. Evidence is found that congenital maldevelopments of nerve tissues occur frequently, and these apparently may disappear, develop into malignant tumors, develop into benign tumors, or even become malignant and then change in whole or in part into nonmalignant tumors. In contrast to the marked heredity factor in retinoblastoma, the other types of tumors that tend to be congenital show no apparent hereditary influence. The significance of congenital malignant growth is discussed in the light of the fact that ordinarily a large part of the life cycle is required to produce any type of malignant tumor.—H. G. W.

Breast

ARCHER, V. W., and G. COOPER. (Univ. of Virginia Hosp., University, Va.) Regression of carcinoma of the breast following artificial menopause. Am. J. Roentgenol., 44:108-109. 1940.

Case report.—H. G. W.

DICKINSON, A. M. (Memorial Hosp., New York, N. Y.) Carcinoma of the axillary tail of the breast. Am. J. Surg., 49:513-517. 1940.

Case report.—H. G. W.

CHARACHE, H. (Brooklyn Cancer Inst., Brooklyn, N. Y.) Neurofibromatosis associated with carcinoma of the breast and pregnancy. Arch. Derm. & Syph., 42:337-340. 1940.

Case report.—H. G. W.

HIDDE, F. G., J. P. Malec, and J. W. GALE. (State of Wisonsin Gen. Hosp., Madison. Wis.) Carcinoma of the breast. Am. J. Surg., 49:427-433. 1940.

Clinical study of outcome in 177 cases.—H. G. W.

HYNES, K. E. (Northern State Hosp., Sedro-Woolley, Wash.) Non-inflammatory nodules in the female breast. Am. J. Surg., 50:597-604. 1940.

General review.—H. G. W.

PECK, W. S., H. K. RANSOM, and F. J. HODGES. (Univ. of Mich. Med. Sch., Ann Arbor, Mich.) Treatment of advanced and recurrent carcinoma of the breast. Am. J. Roentgenol., 44:866-874. 1940.

Study of 920 cases, more than half inoperable, showing that irradiation is the most effective single agent in dealing with advanced and recurrent breast cancer. The carcinoma was bilateral in 0.9% of the cases.—H. G. W.

PELLER, S. (Sch. of Hygiene and Public Health, Johns Hopkins Univ., Baltimore, Md.) Cancer and its relations to pregnancy, to delivery, and to marital and social status. 1. Cancer of the breast and genital organs. Surg., Gynec. & Obst., 71:1-8. 1940.

A study based on the statistics of cancer deaths in England and Wales during 1930-1932. These lead to the conclusion that the distribution of primary neoplasms within the breast and genital organs, and the specific death rates from cancer of each part, are widely influenced by birth rates; the total mortality from cancer of the breast and genital organs, however, varies only to a small degree, as an increased incidence in 1 organ is compensated for by a decrease in incidence in other organs, and vice versa. The lower the social level and the less satisfactory the marital status the earlier the patient manifests cancer. The correlation between cancer and pregnancy in women is entirely different from that in mice.

PELLER, S. (Sch. of Hygiene and Public Health, Johns Hopkins Univ., Baltimore, Md.) Cancer and its relations to pregnancy, to delivery, and to marital and social status. 2. Cancer of organs other than reproductive; total cancer mortality. Surg., Gynec. & Obst., 71:181-186. 1940.

The total cancer mortality as well as the site of the primary tumor is influenced by the marital status. During the age of reproduction married women show higher cancer rates than do spinsters; in the 20 years following there are small differences in favor of married women; above the age of 64 both groups have about equal cancer mortality rates. There is a correlation between the reproductive activity and the changes in the cancer distribution within the genital organs, the breast, biliary system, and stomach, the incidence increasing in some organs and in other diminishing. These changes counterbalance each other. The simultaneous occurrence of cancer with pregnancy seems less frequent than would be expected.—H. G. W.

ROSS, M., and R. G. DORFMAN. (Yale Univ. Sch. of Med., New Haven, Conn.) The urinary excretion of estrogens and androgens by women with carcinoma of the breast. Cancer Research, 1:52-54. 1941.

The urines of 4 women with cancer of the breast were assayed for androgens and estrogens, 3-day samples being used, with 1 exception, when a 2-day sample was used. The quantities of urinary androgens and estrogens excreted did not vary appreciably from those reported for normal females. The findings do not indicate that estrogens and androgens play no role in the etiology of mammary carcinoma; they demonstrate that the disease can be maintained or can progress in the presence of normal amounts of these hormones.—S. B-J.

SHORE, B. R. (St. Luke's Hosp., New York, N. Y.) Carcinoma of the breast. Surg., Gynec. & Obst., 71:515-519. 1940.

Report of 418 cases treated at St. Luke's Hospital, with particular reference to prognosis.—H. G. W.

WARREN, S. (Harvard Med. Sch., Boston, Mass.) The relation of "chronic mastitis" to carcinoma of the breast. Surg., Gynec. & Obst., 71:257-273. 1940.

The development of breast cancer in 1,206 women, whose breasts had been operated upon for benign noninfectous lesions, was studied and compared with the incidence of breast cancer in females of the general population in the same age groups, and it is shown that chronic cystic mastitis and chronic mastitis predispose to the development of breast cancer, but no means exists to determine which of these lesions will develop cancer and which will not. Apparently adenoma is without significance as regards subsequent malignancy, but adenocystoma is a precancerous lesion. The risk is not sufficiently great to warrant bilateral mastectomy in cases of chronic mastitis and chronic cystic mastitis.—H. G. W.

Female Genital Tract

MEIGS, J. V. (Boston, Mass.) Cancer of the ovary. Surg., Gynec., & Obst., 71:44-53. 1940.

Clinical study.—H. G. W.

ODA, T., S. MATSUSHITA, and F. KUROSU. (Path.-anat. Inst. der Med. Fakultät, Keio-Gijuku Univ., Tokio.) Über einen Fall vom Ovarialteratom in Begleitung von Chorio-epitheliom malignum und Karzinom. A case of teratoma of the ovary in association with malignant chorio-epithelioma and carcinoma. Gann, 34:219-224. 1940.

The authors report the case of a girl 11½ years old from whom a large tumor of the right ovary was removed at operation. The mass contained 3 types of tumor, a teratoma, a chorio-epithelioma, and a solid carcinoma of the ovary. The article is illustrated with 10 figures.—S. B-J.

ARNESON, A. N., and H. HAUPTMAN. (Barnard Free Skin and Cancer Hosp., St. Louis, Mo.) Radiation in the treatment of carcinoma of the body of the uterus. J. A. M. A., 114: 29-33. 1940.

Analysis of statistics reported for corpus cancer shows that the best results are obtained from preoperative irradiation followed by hysterectomy. In the attempt to lessen the amount of preexisting infection a preliminary course of x-rays should be used before the insertion of intra-uterine radium.—H. G. W.

AUSTER, L. S., and A. M. SALA. (New York City Cancer Hosp., New York, N. Y.) Causes of death in cancer of the cervix uteri. Surg., Gynec. & Obst., 71:231-239. 1940.

Analysis of 124 autopsied cases, showing that death is most often the result of sepsis.—H. G. W.

BARANY, E., S. BARANY, and M. ANGELO. (New York City Cancer Inst., New York, N. Y.) Squamous cell epithelioma of the cervix uteri with bone metastasis. Am. J. Roentgenol., 44:579-585. 1940.

Report of 2 cases.—H. G. W.

FIENBERG, R. (Westfield State Sanatorium, Westfield, Mass.) Endometrial sarcoma. Arch. Path., 29:800-812. 1940.

Case reports.—H. G. W.

HALL, D. P. (Univ. of Louisville Sch. of Med., Louisville, Ky.) Lipomata of the uterus. Am. J. Surg., 50:119-123. 1940.

The 4th case in Anglo-American literature is reported.—H. G. W.

JOHNSTON, R. A., and G. F. ADAM. (Houston, Texas.) Tumors complicating pregnancy. South. Med. J., 33:845-848. 1940.

There were 47 tumors in approximately 4,500 deliveries, at least ¾ being fibromyomas.—H. G. W.

KAWANAGO, S. (Frauenklinik und klin.-path. Abt. med. Fakultät der Keio-Gijuku Univ., Tokyo.) Über wichtige zytologisch-histologische Befunde beim Uteruskarzinom. The cytology of carcinoma of the uterus. Gann, 34:39-47. 1940.

Cyclic nuclear changes and the formation of endogenous daughter cells within the cells of squamous cell carcinoma of the liver are described and illustrated in 13 figures. One of these (N. Fig. 12), is a schematic representation of the complex mother cell and diagrams of stages in the development of endogenous daughter cells.—S. B-J.

MASSON, J. C., and R. O. GREGG. (Mayo Clinic, Rochester, Minn.) Carcinoma of the body of the uterus. Experience of the Mayo Clinic for twenty-four years. Surg., Gynec. & Obst., 70:1083-1099. 1940.

Clinical study.—H. G. W.

WALSH, G., and R. M. POOL. (Fairfield, Ala.) Similar fibroid tumors occurring in identical twins. South. M. J., 33: 1098-1100. 1940.

Uterine fibroids were removed at about the same time from identical negro twins.—H. G. W.

PLAUT, A., and M. L. DREYFUSS. (Beth Israel Hosp., New York, N. Y.) Adenosis of vagina and its relation to primary adenocarcinoma of the vagina. Surg., Gynec. & Obst., 71:756-765. 1940.

Report of 3 cases and review of the literature. The glands in adenosis of the vagina probably originate from muellerian epithelium which takes part in the formation of the vaginal epithelium, and although they resemble cervical glands they do not arise in the cervix. Primary adenocarcinoma of the vagina probably usually arises in adenosis.—H. G. W.

KILFOY, E. J. (Los Angeles, Calif.) Squamous cell epithelioma of the vulva. Am. J. Surg., 50:114-118. 1940.

Case report.—H. G. W.

Male Genital Tract

CARSON, W. J. (Univ. of Maryland, Baltimore, Md.) Tumors of the penis. J. Urol., 44:307-313. 1940.

Clinical report.—H. G. W.

BARNES, R. W. (Coll. of Med. Evangelists, Los Angeles, Calif.) Carcinoma of the prostate. J. Urol., 44:169-176. 1940.

Statistical analysis of 677 cases with reference to results of treatment.—H. G. W.

COUNSELLER, V. S., and R. E. BEDARD. (The Mayo Foundation, Rochester, Minn.) Sarcoma of the prostate gland. J. Urol., 43:836-843. 1940.

Report of 4 cases.—H. G. W.

FISTER, G. M. (Ogden, Utah.) Sarcoma of the prostate. J. Urol., 44:835-840. 1940.

Case report.—H. G. W.

MARKS, J. H. (Palmer Memorial Hosp., Boston, Mass.) A study of bone metastasis from carcinoma of the prostate. Am. J. Roentgenol., 44:732-736. 1940.

Study of 148 cases. Roentgen examination of 108 cases gave evidence of bone metastasis in 60%. The metastases are predominantly of the osteoplastic type, and in early cases are especially localized in the pelvis.—H. G. W.

QUINLAND, W. S., and J. R. CUFF. (Meharry Med. Coll., Nashville, Tenn.) Primary carcinoma in the negro. Arch. Path., 30:393-402. 1940.

A statistical study of 300 cases, emphasizing the importance of prostatic cancer.—H. G. W.

STEVENS, A. R., and B. S. BARRINGER. Sarcoma of the prostate. J. Urol., 44:83-108. 1940.

Report of 16 cases of prostatic growths, including 3 cases of anaplastic carcinoma.—H. G. W.

DREYFUSS, M. L., and S. LUBASH. (Beth Israel Hosp., New York, N. Y.) Malignant mixed tumor of the spermatic cord (lipo-osteo-fibrosarcoma). J. Urol., 44:314-324. 1940.

Case report.—H. G. W.

BOSSE, M. D. (Western Pennsylvania Hosp. Inst. of Path., Pittsburgh, Penn.) Rhabdomyosarcomatous pulmonary metastases from a teratoma testis. Am. J. Cancer, 39:343-346. 1940.

A case of teratoma testis with bulky pulmonary metastases is described in a 27 year old man. Microscopic examination of the metastases revealed large amounts of striated muscle. The pathological description in the text is supplemented by photomicrographs.—L. L. W.

GILBERT, J. B. (Schnectady, N. Y.) Studies in malignant testis tumors. 2. Syndrome of choriogenic gynecomastia. Report of 6 cases and review of 129. J. Urol., 44:345-357. 1940.

A detailed study of 46 cases establishes a syndrome of choriogenic gynecomastia consisting of stimulation from a primary or metastatic chorionepitheliomatous tumor of the testicle. —H. G. W.

GILBERT, J. B., and J. B. Hamilton. (Yale Univ. Sch. of Med., New Haven, Conn.) Studies in malignant testis tumors. 3. Incidence and nature of tumors in ectopic testes. Surg. Gynec. & Obst., 71:731-743. 1940.

Analysis of 841 cases shows that testicular cancer accounts accounts for 0.6% of male cancer deaths, that patients with testicular cancer frequently exhibit congenital defects, and that there is an association with ectopy 48 times higher than expected by chance. The frequency of bilateral involvement of ectopic testes is 32 times that of scrotal testes. However, the predisposition to testis cancer can be better correlated with congenital conditions than with an ectopic site.—H. G. W.

GORDON, G. (Univ. of Michigan Med. Sch., Ann Arbor, Mich.) Tumors of the testis; a study of the pathology of 142 cases of primary neoplasms of the testis in man. J. Urol., 43:851-858. 1940.

Evidence is presented favoring the hypothesis that these neoplasms are of teratomatous origin.—H. G. W.

KIRSHBAUM, J. D., and M. D. JACOBS. (Cook County Hosp., Chicago, Ill.) Dysgerminoma of the testicle. Surg., Gynec. & Obst., 71:297-301. 1940.

A clinical and pathological study of 30 cases of malignant tumors of the testicle, 26 of which were classified as dysgerminoma. The term dysgerminoma is recommended as describing a distinct group of tumors of the testicle or ovary which heretofore have been known by a variety of terms.—H. G. W.

MELICOW, M. M. (Columbia Univ. Coll. of Med., New York, N. Y.) Embryoma of testis. Report of case and a classification of neoplasms of the testis. J. Urol., 44:333-340. 1940.

A report of an unusual testicular tumor containing innumerable embryos.—H. G. W.

STEVENS, W. E. (San Francisco, Calif.) Benign teratomas (dermoid cysts) of the testicle. J. Urol., 44:864-870. 1940.

Case report.—H. G. W.

Urinary System—Male and Female

ASH, J. E. (Army Med. Museum, Washington, D. C.) Epithelial tumors of the bladder. J. Urol., 44:135-145. 1940.

An analysis of 2,500 cases collected by the Bladder Tumor Registry of the American Urological Association.—H. G. W.

HAMM, F. C. (Bushwick Hosp., Brooklyn, N. Y.) Benign cystadenoma of the bladder, probably of urachal origin. J. Urol., 44:227-233. 1940.

Case report.—H. G. W.

THOMPSON, G. J., and J. R. McDONALD. (Mayo Clinic, Rochester, Minn.) Benign tumors of the urinary bladder: Report of a case of neurofibroma. J. Urol., 43:831-835. 1940.

Clinical report.—H. G. W.

BOSSE, M. D. (Western Pennsylvania Hosp., Pittsburgh, Penn.) Glomerulus-like neoplastic structures in a carcinoma of the kidney. Arch. Path., 30:1235-1239. 1940.

Case report with analysis of the literature, leading to the conclusion that the term "glomeruloma" is not justified as a designation of a renal tumor showing glomerulus-like structures, for similar structures may be found in tumors of other organs.—H. G. W.

HAYWARD, W. G. (Jamestown, N. Y.) Malignant papillary cystadenoma of the kidney. J. Urol., 44:259-263. 1940.

Case report.—H. G. W.

HECKEL, N. J., and H. V. GOULD. (Ravenswood Hosp., Chicago, Ill.) A report of a papillary cystadenoma of the kidney. J. Urol., 44:200-205. 1940.

KOZOLL, D. D., and J. D. KIRSHBAUM. (Cook County Hosp., Chicago, Ill.) Relationship of benign and malignant hypernephroid tumors of kidney. Clinical and pathological study of 77 cases in 12,885 necropsies. J. Urol., 44:435-449. 1940.

The great frequency of multiple histological types and structures, including sarcomatous tendencies, is emphasized.—H. G. W.

SCHOLL, A. J. (Los Angeles, Calif.) Squamous cell changes and infection of the urinary tract. J. Urol., 44:759-767. 1940.

Squamous cell changes followed long standing urethral obstruction and infection, with carcinoma in 1 of 2 cases.—H. G. W.

Oral Cavity and Upper Respiratory System

FIGI, F. A. (Mayo Clinic, Rochester, Minn.) Fibromas of the nasopharynx. J. A. M. A., 115:665-671. 1940.

Clinical study of 63 cases of juvenile basal fibroma of the nasopharynx.—H. G. W.

GOTSHALK, H. C., C. F. TESSMER, and J. W. SMITH. (Queen's Hosp. Tumor Clinic, Honolulu, Hawaii.) Malignant melanoma of the palate. Arch. Path., 30:762-765. 1940.

Report of a case of widespread malignant melanoma originating in the region of the hard palate.—H. G. W.

HANAFUSA, S., and K. KUBOTA. (Path Inst. u. Otorhinolaryngol. Klin. der Keio-Gijuku Univ., Tokyo.) Über einen Fall von Reticulosarkom des Rachens. A case of reticulosarcoma of the pharynx. Gann, 34:231-238. 1940.

The clinical history and findings at necropsy are reported in the case of a man 43 years old who had a relatively localized reticulosarcoma of the right side of the pharynx, with metastases in the cervical lymph nodes and in both kidneys. The report is illustrated with 6 figures.—S. B-J.

LIVINGSTON, S. K. (Hines, Ill.) Teratoma (mixed tumor) of the right parotid gland with metastasis to sternum and second rib left. Am. J. Roentgenol., 44:887-888. 1940.

Case report.—H. G. W.

MARTIN, H. E., and E. L. SUGARBAKER. (Memorial Hosp., New York, N. Y.) Cancer of the floor of the mouth. Surg., Gynec. & Obst., 71:347-359. 1940.

Clinical study of 103 cases.—H. G. W.

STEWART, H. L., and M. M. LIEBER. (From the National Cancer Inst., Bethesda, Md. and the Dept. of Path., Jefferson Med. Coll., Philadelphia, Penn.) Squamous cell carcinoma of the Eustachian tube. Arch. Path., 30:518-532. 1940.

A clinical and pathological study of a case with review of the literature.—H. G. W.

Intrathoracic Tumors—Lungs—Pleura

BRUNN, H. and A. GOLDMAN. (Univ. of California Med. Sch., San Francisco, Calif.) The differentiation of benign from malignant polypoid bronchial tumors. Surg., Gynec. & Obst., 71:703-722. 1940.

Clinical study.—H. G. W.

CARTER, B. N. (Coll. of Med., Univ. of Cincinnati, Cincinnati, Ohio.) Resection of a portion of the thoracic esophagus for carcinoma. Report of two cases. Surg., Gynec. & Obst., 71:624-632. 1940.

Clinical report.—H. G. W.

CASILLI, A. R., and H. J. WHITE. (James S. Green Tumor Clinic, Elizabeth, N. J., and the Roosevelt Hosp. for Tuberculosis, Middlesex County, N. J.) Rare forms of primary malignant lung tumors. Am. J. Clin. Path., 10:621-641. 1940.

Report of 3 cases, namely: malignant adenoma from bronchial mucous glands; malignant adenoma from alveolar ducts; carcinomatoides alveogenica multicentrica from the alveolar cell lining.—H. G. W.

FARIÑAS, P. L. (Cancer Inst., Havana, Cuba.) Recent progress in the bronchographic examination of bronchogenic carcinoma. Am. J. Roentgenol., 44:370-385. 1940.

The bronchographic examination showing the location and extension of the neoplastic process may serve as a guide in determining whether pneumectomy or lobectomy is indicated.—H. G. W.

HALL, W. C. (Temple Univ. Hosp., Philadelphia, Penn.) The origin of tumors occurring in the apex of the lung. Am. J. Roentgenol., 44:838-847. 1940.

Review of literature and study of 5 cases shows that there is no specific tumor in the apex of the lung which will give characteristic signs and symptoms to the exclusion of other types of cancer.—H. G. W.

HAMILTON, C. E., and N. H. WEXLER. (Kings County Hosp., Brooklyn, N. Y.) Carcinoma and tuberculosis of the lung. Am. Rev. Tuberc., 42:334-342. 1940.

Case report.—H. G. W.

JACOBSON, V. C. (Albany Med. Coll., Albany, N. Y.) The deleterious effects of deep roentgen irradiation on lung structure and function. Am. J. Roentgenol., 44:235-249. 1940.

Case report of a patient surviving x-ray therapy for carcinoma of the lung for 16 years.—H. G. W.

LEDDY, E. T., and H. J. MOERSCH. (Mayo Clinic, Rochester, Minn.) Roentgen therapy for bronchogenic carcinoma. J. A. M. A., 115:2239-2242. 1940.

Report of 250 cases, 25 of which given irradiation have survived from 1 to 12 years.—H. G. W.

LOURIA, M., M. LEDERER, and L. HERZ. (Jewish Hosp. of Brooklyn, Brooklyn, N. Y.) Neurofibromatosis with sarcomas in the lungs. J. Thoracic Surg., 9:612-622. 1940.

Case report.—H. G. W.

MACKLIN, M. T., and C. C. MACKLIN. (Univ. of Western Ontario Med. Sch., London, Ontario.) Does chronic irritation cause primary carcinoma of the human lung? Arch. Path., 30:924-955. 1940.

General review, with the conclusion that it is impossible to assign to any extrinsic factor an absolute role in carcinogenesis in any individual case.—H. G. W.

McCORKLE, R. G., C. J. KOERTH, and J. M. DONALDSON, JR. (Woodman of the World Memorial Hosp., San Antonio, Texas.) Thoracic lipomas. J. Thoracic Surg., 9:568-582. 1940.

Report of an intrathoracic lipoma weighing 16 pounds, with review of the literature.—H. G. W.

OLDS, J. W., and B. R. KIRKLIN. (Mayo Clinic, Rochester, Minn.) Primary carcinoma of the lung. Am. J. Roentgenol., 44:357-369. 1940.

A roentgenological study of 206 proved cases, in which a definite diagnosis was made or suggested in about 60%.—H. G. W.

PHILIPS, B. (Montefiore Hosp., New York, N. Y.) Intrathoracic pheochromocytoma. Arch. Path., 30:918-921. 1940.

Case report of the 11th tumor of this sort arising unassociated with the adrenal. The tumor was situated at the apex of the left pleural cavity, suggesting a superior pulmonary sulcus syndrome, and was associated with marked cardiorenal disease.—H. G. W.

STRYKER, W. A. (Univ. of Chicago, Chicago, Ill.) Leiomyosarcoma of the pleura. Arch. Path., 30:750-754. 1940.

Report of the 2nd case, and the 1st with metastases.—H. G. W.

TRIPOLI, C. J., and L. F. HOLLAND. (Sch. of Med. of Louisiana State Univ., New Orleans, La.) Carcinoma of the lung. An analysis of 195 cases with a special note on needle puncture biopsy. South. M. J., 33:559-564. 1940.

Clinical study.—H. G. W.

VAN HAZEL, W. (Univ. of Illinois Coll. of Med., Chicago, Ill.) Joint manifestations associated with intrathoracic tumors. J. Thoracic Surg., 9:495-504. 1940.

Hypertrophic pulmonary osteoarthropathy may be the initial symptom in intrathoracic tumors, especially if the blood vessels are involved.—H. G. W.

Gastrointestinal Tract

ABRAHAMSON, R. H., and J. W. HINTON. (New York, N. Y.) Carcinoma of the stomach. Review of 444 cases to emphasize the inadequacy of present methods for an early diagnosis. Surg., Gynec. & Obst., 71:135-141. 1940.

Clinical study.—H. G. W.

BARRETT, N. R. (London, England.) Fibroma of the esophagus. J. Thoracic Surg., 9:672-678. 1940.

Case report.—H. G. W.

BENJAMIN, E. L., and F. CHRISTOPHER. (Evanston Hosp., Evanston, Ill.) Primary lymphosarcoma of the small intestine. Am. J. Clin. Path., 10:408-413. 1940.

Case report.—H. G. W.

BRODERS, A. C., L. A. BUIE, and D. R. LAIRD. (Mayo Clinic, Rochester, Minn.) Prognosis in cancer of the rectum. J. A. M. A., 115:1066-1071. 1940.

A comparison of the Broders and Dukes method of classification on 432 resected specimens of carcinoma of the rectum, with the conclusion that a combination of the 2 yields a more accurate prognosis than either alone.—H. G. W.

CLARKE, B. (Rhode Island Hosp., Providence, R. I.) Myoepithelial hamartoma of the gastrointestinal tract. Arch. Path., 30:143-152. 1940.

A study of 8 cases together with a review of the literature indicate that the diversity of histologic structure is dependent on degree of differentiation rather than point of origin or original cell type.—H. G. W.

CYTRONBERG, S. (Mexico City.) Early diagnosis of gastric cancer. Rev. Gastroenterol., 7:391-399. 1940.

Refractometric examination of the stomach contents is recommended.—H. G. W.

DOUB, H. P. (Henry Ford Hosp., Detroit, Mich.) The differential diagnosis of pyloric and prepyloric ulceration. Am. J. Roentgenol., 43:826-831. 1940.

In all chronic indurated ulcerated lesions there will be a probable occurrence of malignant neoplasm in from 15 to 30%—H. G. W.

DUBLIN, W. E., R. O. GREGG, and A. C. BRODERS. (Mayo Clinic, Rochester, Minn.) Mitosis in specimens removed during day and night from carcinoma of the large intestine. Arch. Path., 30:893-895. 1940.

Specimens of 5 adenocarcinomas of the colon taken during the morning and night showed the same glandular pattern, degree of differentation, and mitosis coefficient.—H. G. W.

EWING, J. (Memorial Hosp., New York, N. Y.) Etiological indications of early gastric cancer. Rev. Gastroenterol., 7:305-309. 1940.

Little progress has been made in detecting the exciting causes of gastric cancer. Statistics showing that the incidence of gastric cancer is related to dietary habits tend to indicate that the disease is dependent upon external causes and is therefore preventable. But the exact nature of the external factors is not known. The doctrine of chronic irritation as a cause of gastric cancer has become unsatisfactory since the recognition of carcinogens. Carcinogenic chemicals may be involved but it may be found that the earliest stages of gastric cancer are incompatible with the idea that any one set of agents or any one method of origin will apply to this common form of cancer. Constitutional factors and heredity may be predisposing factors. Six bases for the development of gastric cancer are discussed. These are: congenital abnormalities of the stomach; polypoid adenomas; pernicious anemia with atrophic gastritis; peptic ulcer; buccal infection; malignant change of the chief cells lining the fundus of the glands. The consensus of opinion appears to be that chronic indurated peptic ulcer seldom becomes transformed into cancer. Difficulties of determining whether a case presents ulcer or cancer or both are discussed.—S. B-J.

GAINEY, J. J., and L. M. FRIEDLAND. (Cumberland Hosp., Brooklyn, N. Y.) Adenocarcinoma of the jejunum, treated by enteroenterostomy and complicated by intussusception. Am. J. Surg., 50:112-113. 1940.

Case report.—H. G. W.

GRAY, H. K. (Mayo Clinic, Rochester, Minn.) Carcinoma of the stomach. With particular reference to the significance of persistent symptoms ascribed to the stomach and the malignant potentiality of gastric ulcers. J. A. M. A., 116:22-25. 1940.

Case report.—H. G. W.

KAPLAN, I. I., and S. RUBENFELD. (Bellevue Hosp., New York, N. Y.) Carcinoma of the anus. Its incidence, pathology and radiation treatment. Am. J. Roentgenol., 44:265-269. 1940.

Clinical report of 8 cases.—H. G. W.

LAMSON, O. F. (Seattle, Wash.) Multiple primary carcinoma. Surg., Gynec. & Obst., 71:650-653. 1940.

Two cases. 1. Carcinoma of the colon and breast. 2. Carcinoma of the cecum and ovary.—H. G. W.

MALLORY, T. B. (Massachusetts Gen. Hosp., Boston, Mass.) Carcinoma in situ of the stomach and its bearing on the histogenesis of malignant ulcers. Arch. Path., 30:348-362. 1940.

The noninvasive stage of cancer of the stomach is described and illustrated on the basis of 4 cases in which nonpolypoid lesions were associated with peptic ulceration. Histologic evidence is presented that secondary peptic ulceration of cancerous tissue in the stomach is not uncommon, and the validity of histologic criteria has been confirmed by roentgenologic evidence of dimunition in the size of the ulcer crater under medical treatment in 4 cases. The significance of these findings in relation to the genesis of the malignant ulcer is discussed.—H. G. W.

MASS, M., and J. D. KIRSHBAUM. (Cook County Hosp., Chicago, Ill.) Leiomyosarcoma of the fundus of the stomach with perforation. Am. J. Roentgenol., 44:716-718. 1940.

Case report.—H. G. W.

ROSS, D. E. (Ross-Loos Med. Group, Los Angeles, Calif.) Sarcoma of the stomach. Am. J. Surg., 49:503-507. 1940.

Case report.—H. G. W.

SCHENKEN, J. R., J. STASNEY, and W. K. HALL. (Sch. of Med., Louisiana State Univ., New Orleans, La.) The antianemic principle in the human liver in carcinomas of the stomach and cecum. Am. J. M. Sc., 200:11-17. 1940.

The antianemic principle was not present in the liver in 1 case of carcinoma of the pylorus, but present in the livers of 3 patients with carcinoma not involving the pylorus.—H. G. W.

SCHINDLER, R. (Univ. of Chicago, Chicago, Ill.) Early diagnosis and prognosis of gastric carcinoma. J. A. M. A., 115:1693-1699. 1940.

Clinical study, with emphasis on routine x-ray and gastroscopic examinations.—H. G. W.

SCHINDLER, R., P. B. NUTTER, H. E. GROOM, and W. L. PALMER. (Univ. of Chicago, Chicago, Ill.) Anatomic foundation of anacidity, a gastroscopic study. Arch. Int. Med., 66: 1060-1078. 1940.

Among 101 patients having spontaneous histamine-proved anacidity were 23 with malignant gastric tumor, all but 3 of whom had gastritis. This is in accord with the concept that atrophic gastritis plus anacidity may precede the formation of gastric carcinoma and that in many cases severe atrophic gastritis may be responsible for the anacidity. In the 3 cases without gastritis there was so much ulceration as to suggest that toxic material from the growth inhibited the secretion (Brunschwig). —H. G. W.

SMITH, T. E. (Baylor Univ. Coll. of Med., Dallas, Texas.) The grading of rectal cancer. South. Med. J., 33:593-599. 1940.

Clinical study, recommending a combination of Dukes' and Broders' methods.—H. G. W.

SCHWEIGER, L. R., and J. A. BARGEN. (Mayo Clinic, Rochester, Minn.) Multiple primary malignant lesions of the large bowel. Arch. Int. Med., 66:1331-1340. 1940.

A study of 23 cases.—H. G. W.

WALTERS, W. (Mayo Clinic, Rochester, Minn.) The surgical treatment of ulcerating gastric lesions. Am. J. Roentgenol., 43:819-825. 1940.

Experience has shown that the report of the roentgenologist that a gastric ulcer is malignant is practically always correct, but the report of a benign ulcer does not always exclude malignancy. Accumulating evidence indicates that the percentage of gastric ulcers that are malignant as well as the number of cancers that start as gastric ulcers is nearer 20 than 10%—H. G. W.

WOODRUFF, R., and J. R. McDONALD. (Mayo Foundation and Clinic, Rochester, Minn.) Benign and malignant cystic tumors of the appendix. Surg., Gynec. & Obst., 71:750-755. 1940.

In appoximately 43,000 appendixes removed surgically were found 136 benign cystic tumors and 10 cases of cystic adenocarcinomas which had apparently arisen in the wall of a benign mucocele. The benign mucoceles are the result of a stricture, usually inflammatory.—H. G. W.

Bone and Bone Marrow

DeSANTO, D. A., and E. BURGESS. (Hosp. for Ruptured and Crippled, New York, N. Y.) Primary and secondary neurilemmoma of bone. Surg., Gynec. & Obst., 71:454-461. 1940.

Report of 2 cases of this rare tumor, which is not to be confused with the neurofibromas.—H. G. W.

EDEIKEN, L. (Mount Sinai Hosp., Philadelphia, Penn.) Benign giant cell tumor of bone. Am. J. Roentgenol., 44: 384-886. 1940.

A case reported of 5 year cure by irradiation alone.—H. G. W.

FERGUSON, A. B. (New York Orthopaedic Dispensary and Hosp., New York, N. Y.) Treatment of osteogenic sarcoma. J. Bone & Joint Surg., 22:916-922. 1940.

Recommends delay of amputation with the use of radiation, or excision, or both, before amputation.—H. G. W.

ITOH, M., and K. MAKINO. (Path. Inst. u. Chirurg. Abt. Staatlichen Zahnärztlichen Hochschule, Tokio.) Ein Sectionsfall von Ewing-Sarkom (Retikulosarkom des Knochenmarks). Report of necropsy in a case of Ewing's reticulosarcoma of the bone marrow. Gann, 34:339-345. 1940.

This is a brief summary of the clinical history and the findings at necropsy in the case of a boy, 6 years old, who died 6 months after the beginning of the growth of a tumor of the right mandible. The tumor was diagnosed as Ewing's reticulosarcoma. Metastases were widespread. The differential diagnosis is discussed.—S. B-J.

JAFFE, H. L., L. LICHTENSTEIN, and N. B. PORTIS. (Hosp. for Joint Diseases, New York, N. Y.) Giant cell tumor of bone. Its pathologic appearance, grading, supposed variants and treatment. Arch. Path., 30:993-1031. 1940.

A stricter conception of what constitutes a true giant cell tumor of bone than has been current is proposed. It is a neoplasm of a definite kind, arising apparently from the undifferentiated supporting connective tissue of the marrow and clearly delimitable on the basis of its cytological details. It is composed of a more or less vascularized network of spindle shaped cells or ovoid stromal cells and multinucleated giant cells in certain particular proportions and arrangements, and shows but little collagenous differentiation of its stoma and almost no evidence of ossification. On the basis of difference in aggressiveness the giant cell tumors are subclassified into 3 grades.—H. G. W.

KEEGAN, J. J., and C. P. BAKER. (Omaha, Neb.) Giant cell tumor of the frontal bone. J. Lab. & Clin. Med., 26: 319-322. 1940.

Report of a case, which is unusual in membranous bone.—H. G. W.

SCHWARTZ, C. W. (Neurological Inst., New York, N. Y.) Cranial osteomas. Am. J. Roentgenol., 44:188-196. 1940.

A discussion of 48 cases.—H. G. W.

WAUGH, J. M. (Mayo Clinic, Rochester, Minn.) Fibroma of the musculofascial layers of the abdominal wall (Desmoid tumors). Am. J. Surg., 50:694-697. 1940.

Desmoid tumors in many instances apparently arise secondary to trauma, and probably are related to a disturbance in the organization of the ensuing hematoma. They are probably benign.—H. G. W.

KEKWICK, R. A. (Lister Inst., London, England.) Biochem. J., 34:1248-1257. 1940.

Sera from 5 cases have been submitted to ultracentrifugal and electrophoretic analysis. Four of the sera showed the same 3 components as normal serum with the globulin component quantitatively very much increased. The 5th specimen however contained 5 different protein constituents. Previous work of this type has shown that sera of many myelomatous cases contain 3 proteins while in 1 case 4 components were described. (McFarlane. Biochem. J., **29**:1175. 1935.) Bence-Jones proteinuria was not observed in any of the 5 cases.—E. B.

KING, B. B. (New York, N. Y.) Solitary plasma cell myeloma of bone as an initial stage of multiple myeloma. J. A. M. A., 115:36-38. 1940.

Case report.—H. G. W.

MARQUIS, W. J. (Newark, N. J.) The correlation of the roentgen and blood examinations in multiple myeloma. Am. J. Roentgenol., 44:858-865. 1940.

A study of the blood chemistry may give information which, together with the roentgen examination, will establish a diagnosis.—H. G. W.

Muscle and Tendon

BRUNSCHWIG, A. and D. TSCHETTER. (Univ. of Chicago, Chicago, Ill.) Experiences in the treatment of primary skeletal sarcoma by irradiation. Am. J. Roentgenol., 44:83-89. 1940.

Report of 20 cases treated by irradiation with clinical cure for 3 years in 6.—H. G. W.

LEWIS, R. W. (Hosp. for Ruptured and Crippled. New York, N. Y.) Roentgen recognition of synovioma. Am. J. Roentgenol., 44:170-174. 1940.

Four cases are described and illustrated in which distinctive and essentially identical roentgenograms were found to indicate malignant, or potentially malignant, synoviomas or synovial sarcomas.—H. G. W.

Leukemia—Lymphosarcoma—Hodgkin's Disease

BAGGENSTOSS, A. H., and F. J. HECK. (Mayo Clinic, Rochester, Minn.) Follicular lymphoblastoma. (Giant lymph follicle hyperplasia of lymph nodes and spleen.) Am. J. M. Sc., 200:17-27. 1940.

A study of 13 cases and of 59 from the literature, leading to the conclusion that it is a connecting link between systemic hyperplasia of the lymphatic system and lymphosarcomatosis. It has a relatively benign early course but is eventually fatal.—H. G. W.

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Confirmation of the observation of Koster that the sedimentation rate remains level through a period of 24 hours. Of 118 malignancies the results were positive in 95.7% and negative in 94.6% of nonmalignant disease.—H. G. W.

LAMB, J. H., and H. A. STOUT. (Oklahoma City, Okla.) Reticulo-endotheliosis. South. M. J., 33:1117-1125. 1940.

Three cases of different phases of neoplastic reticulo-endotheliosis are presented with emphasis on their close genetic interrelationship.—H. G. W.

GOLDBERG, S. A. (Presbyterian Hosp., Newark, N. J.) Primary splenic neoplasms. Am. J. Clin. Path., 10:700-707. 1940.

Report of 2 lymphangiomas and 1 hemangioma.—H. G. W.

SCHAAF, R., and M. KRAEMER. (Presbyterian Hosp., Newark, N. J.) Lymphosarcoma of the ileocaecal valve. Rev. Gastroenterol., 7:248-251. 1940.

Case report.—H. G. W.

SUGARBAKER, E. D., and L. F. CRAVER. (Memorial Hosp., New York, N. Y.) Lymphosarcoma. J. A. M. A., 115: 17-23, 112-116. 1940.

A study of 196 cases with biopsy.—H. G. W.

WATSON, W. L., and W. D. McCARTHY. (Memorial Hosp., New York, N. Y.) Blood and lymph vessel tumors. Surg., Gynec. & Obst., 71:569-588. 1940.

On the basis of a study of 1,056 patients with 1,363 individual blood and lymph vessel tumors a classifications is suggested and methods of therapy are appraised.—H. G. W.

Adrenal

BRUNSCHWIG, A., and E. Humphreys. (Univ. of Chicago, Chicago, Ill.) Excision of pheochromocytoma following near fatal attack of paroxysmal hypertension. J. A. M. A., 115: 355-357. 1940.

Case report.—H. G. W.

MELICOW, M. M., and H. H. GILE. (Columbia Univ. Sch. of Med., New York, N. Y.) An hypernephroma in a polycystic kidney: Review of literature and report of a case. J. Urol., 43:767-773. 1940.

The first complete report.—H. G. W.

ZUCKERMAN, S. S., and H. E. STUCKENHOFF. (Cook County Hosp., Chicago, Ill.) Virilism associated with adrenal cell-rest tumor. Am. J. Clin. Path., 10:822-829. 1940.

Case report.—H. G. W.

Pancreas

LLOYD, T. P., and W. F. DRUMMOND. (Highland Clinic, Shreveport, La.) Carcinoma of the pancreas: With a clinical review of eight cases. New Orleans M. & S. J., 93:223-227. 1940.

Thyroid

WATSON, W. L., and J. L. POOL. (Memorial Hosp., New York, N. Y.) Surg., Gynec. & Obst., 70:1037-1050. 1940.

Clinical study of 167 cases of carcinoma and 5 cases of lymphosarcoma of the thyroid.—H. G. W.

STASTISTICS

ANON. Cancer mortality in the United States. A Review. Pub. Health Rep., 55:1541-1543. 1940.

This is a review of Public Health Bulletins Nos. 248 and 252, issued in 1939 and 1940, respectively, as "the first in a series of studies of cancer mortality in the United States made from unpublished data made available by the United States Bureau of the Census." The annual records of deaths from cancer afford a large body of data which are of value with respect to a study, particularly of the trend and geographic distribution of cancer.

Bulletin No. 248, by Associate Statistician Mary Glover, is entitled "Trend of recorded cancer mortality in the death registration States from 1900 to 1935." Bulletin No. 252, by the same author, is entitled "Recorded cancer mortality in geographic sections of the death registration States from 1920 to 1935."—S. B-J.

GAFAFER, W. M. (Nat. Inst. of Health, U. S. Pub. Health Service.) Disabling morbidity, and mortality from cancer among the male employees of an oil refining company with reference to age, site, and duration, 1933-38, inclusive. Pub. Health Rep., 55:1517-1526. 1940.

The paper analyzes 70 cases of cancer among the male members of the sick benefit plan of an oil refinery during the 6 years, 1933-38. The exposure comprises approximately 60,000 years of life for male employees. There were 46 deaths. The exposure yielded a death rate of 0.78 per 1,000 and a frequency of 1.2 per 1,000. Almost 70 per cent of the deaths were related to the digestive system. The 2 sites, stomach and other abdominal organs accounted for more than half of the cases. Each specific site showed increases with age with respect to both frequency and mortality. For all ages the ratio of cases to deaths was less than 1.2 in 3 sites, esophagus, stomach, and other abdominal organs. Cancer of the lung showed the longest average case duration as well as the longest average duration of nonfatal cases.—Author's summary.

McDOWELL, A. J. (Nat. Inst. of Health, U. S. Pub. Health Service.) The incidence of cancer in Pittsburgh and Allegheny County, Pennsylvania, 1937. Pub. Health Rep., 55:1419-1451. 1940.

This is the third of a series of papers giving the findings of a sampling survey of cancer incidence and prevalence in the United States. It presents the detailed analysis of statistics of 6,103 cases either under medical care, or dying of cancer during one calendar year, 1937 for some cities, and 1938, for others, in Pittsburgh and Allegheny County. The reports indicated a case rate of 319 per 100,000. The 3 most important primary sites among males were digestive tract, genito-urinary system, and skin; for females, genito-urinary system, breast and digestive tract. Malignant neoplasms of these sites constituted 70% of the cases among males and 79% of those among females. Great differences were found to exist between the relative fatality of malignant growths occurring in various sites. The brain, digestive tract, and respiratory system were the sites with the lowest ratio of cases to deaths.—S. B-J.

PARRAN, T. (U. S. Public Health Service), and W. L. AUSTIN (U. S. Bureau of the Census). A diagnosis code for use in tabulating morbidity statistics. Pub. Health Rep., 55: 1558-1575. 1940.

This list was drawn up by a special committee to meet the need for a uniform tabular list for morbidity statistics that was closely linked to the last revision of the International List of Causes of Death. Section II gives the list of diagnosis categories for neoplasms.—S. B-J.

CANCER CONTROL AND PUBLIC HEALTH

BRYAN, E. R. (U. S. Pub. Health Service.) The Tumor Clinic of the Baltimore Marine Hospital. Pub. Health Rep., 55:2195-2199. 1940.

The report of patients treated is illustrated with pictures of new apparatus for x-ray and radium therapy.—S. B-J.

ROSAHN, P. D. (New Britain Gen. Hosp., New Britain, Conn.) The tumor clinic of the New Britain General Hospital and survey of 1939 experience. Bull. Am. Coll. Surgeons, 25: 247-251. 1940.

The organization and conduct of the New Britain General Hospital and Tumor Clinic are described.—H. G. W.

VOEGTLIN, C., and R. R. SPENCER. (National Cancer Inst., Bethesda, Md.) The federal cancer control program. J. Nat. Cancer Inst., 1:1-9. 1940.

After describing federal organizations and policies in general, the research program of the National Cancer Institute is given. The work consists of the following main projects:

(1) Lung cancer, histogenesis, genetic, and chemical predisposing factors; (2) skin cancer, predisposing and protective factors; (3) gastric cancer, induced by carcinogenesis; (4) genetic studies, particularly those relating to the factor in milk controlling the incidence of breast cancer in mice; (5) synthesis and testing of chemicals related to natural steroids; (6) mode of action of cancer-producing chemicals; (7) therapeutic studies —biological action of high energy radiations; (8) epidemiological studies; (9) clinical research, in collaboration with the tumor clinic of the Marine Hospital, Baltimore, Maryland.—L. L. W.

SCIENTIFIC SOCIETIES AND RESEARCH ORGANIZATIONS

ANON. Journal of the National Cancer Institute. Pub. Health Rep., 55:1831-1832. 1940.

Announcement is made of the first issue of the Journal of the National Cancer Institute and of the purposes and plan of the journal. A review of certain known facts and problems of cancer research is presented briefly.—S. B-J.

ANON. Unit on gerontology in the National Institute of Health. Pub. Health Rep., 55:2099-2101. 1940.

Announcement of a new unit for research into some of the many problems of aging.—S. B-J.

Proceedings of the American Association for Cancer Research. Cancer Research, 1:71-74. 1941.

Summary of meetings of the Council and business sessions of the Association, together with the scientific program, of the 33rd annual meeting held at Pittsburgh, Penn., March 19-20, 1940.—S. B-J.

Proceedings of the 32nd Scientific Meeting of the Japanese Foundation for Cancer Research. Gann, 34:78-214. 1940.

Titles and abstracts of 57 pages presented at this meeting held at the Tokyo Imperial University on April 3, 1940.—S. B-J.